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New England Aquarium, Boston, MA

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A SURVEY OF THE TOXICITY AND CHEMICAL COMPOSITION OF USED DRILLING MUDS

by

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New England Aquarium
Boston, Massachusetts 02110

Project CR806776

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16. ABSTRACT

Chemical characterization and toxicity of oil drilling fluids were investigated by the Edgerton Research Laboratory from 1 October 1979 to August 1983 as part of a comprehensive research program sponsored by the U.S. Environmental Protection Agency (EPA) to determine fate and effects of such fluids in the marine environment. Drilling muds used in the research were supplied by the EPA, the Petroleum Equipment Suppliers Association (PESA), and the American Petroleum Institute (API). The drilling muds were designated "May 15," "May 29," "Sept. 4," "Exxon," "Gilson," "Mobile Bay," "Jay Field," and "PESA." Investigations during the first year centered on the chemical composition and the acute toxicity of drilling muds, and the effects of drilling muds on the recruitment of benthic organisms. In the second year, studies focused on toxicity testing with planktonic copepods, chemical characterization of the toxicity test phases, bioaccumulation studies, and the effects of muds on larval and adult benthic organisms. Investigations during the third and fourth year examined sublethal effects of drilling fluids on clam larvae, trace metal and organic constituents in both drilling fluids and toxicity test-phases, and the preliminary development of a drilling fluid solid phase toxicity test.

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms.
- the effects of toxic organics on ecosystems processes and components.
- the significance of chemical carcinogens in the estuarine and marine environments.

This report summarizes findings on the impact of drilling fluids (muds) on selected marine organisms and the chemical composition of several fluids. These data provide needed information on the effects of used drilling fluids on the clam, *Mercenaria mercenaria*, and other marine organisms and relates, where possible, effects to components of the drilling fluid. Results of this research will provide the regulatory arm of the Agency, and others, an additional data base on the fate and effects of drilling fluids that can be applied to the permitting process.

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ABSTRACT

Chemical characterization and toxicity of oil drilling fluids were investigated by the Edgerton Research Laboratory from 1 October 1979 to August 1983 as part of a comprehensive research program sponsored by the U.S. Environmental Protection Agency (EPA) to determine fate and effects of such fluids in the marine environment. Drilling muds used in the research were supplied by the EPA, the Petroleum Equipment Suppliers Association (PESA), and the American Petroleum Institute (API). The drilling muds were designated "May 15," "May 29," "Sept. 4," "Exxon," "Gilson," "Mobile Bay," "Jay Field," and "PESA." Investigations during the first year centered on the chemical composition and the acute toxicity of drilling muds, and the effects of drilling muds on the recruitment of benthic organisms. In the second year, studies focused on toxicity testing with planktonic copepods, chemical characterization of the toxicity test phases, bioaccumulation studies, and the effects of muds on larval and adult benthic organisms. Investigations during the third and fourth year examined sublethal effects of drilling fluids on clam larvae, trace metal and organic constituents in both drilling fluids and toxicity test-phases, and the preliminary development of a drilling fluid solid phase toxicity test. Toxic components of the used drilling muds tested were present as dissolved components or associated with very slowly settling particles. Some used drilling muds contained lipophilic fractions that were similar to hydrocarbons found in #2 fuel oil in the liquid fraction and suspended particulates fraction and contained #2 fuel oil in whole muds. Muds that contained those components were more toxic than those that did not. Juvenile copepods (Acartia tonsa) were not more sensitive to toxic drilling mud solutions than adults of this species. In general, Cancer irroratus larvae appeared to exhibit toxicity responses to drilling muds that were similar to the copepods tested. Arrested shell development induced by exposure to drilling muds appeared to be a sensitive indicator of stress in bivalve larvae. Total chromium concentration showed no correlation to toxicity in the drilling muds that were tested; however, the highest concentrations of Cr(VI), the most biologically toxic form of chromium, occurred in the test phases that exhibited the greatest toxicity to Mercenaria mercenaria larvae. The muds designated "May 15" and "Sept. 4" appeared to be relatively non-toxic to Pseudopleuronectes americanus and to Menidia menidia, although the "May 15" mud was toxic to Neomysis americana and to Acartia tonsa. A study of the effects of drilling mud on invertebrate recolonization of defaunated sediment showed that recolonization decreased in drilling mud layered on top of sediment when the muds were mixed with sediments. Capitella capitata was much more numerous in recolonization sediments that contained drilling mud. Test results showed that the methods used to prepare drilling mud test media affect the apparent toxicity of the muds.

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OVERVIEW - CONCLUSIONS

Drilling muds used in this research were supplied by the Gulf Breeze Environmental Research Laboratory (EPA) and the Petroleum Equipment Suppliers Association (PESA), and by the American Petroleum Institute (API). The muds were designated as follows: muds from Mobile Bay were called "May 15", "May 29", and "Sept. 4"; additional muds from API and PESA were labelled "Exxon", "Gilson", "Mobile Bay", "Jay Field", and "PESA". The conclusions listed below are meant to provide general overview statements concerning the findings of a series of research projects. Due to the complexity and the diversity of the tests that were conducted, the final report and progress reports should be consulted for the specific criteria and conditions of each test.

1. Toxic components of the muds that were tested were present as very slowly settling species. For most of the elements analyzed (Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) suspended/dissolved concentrations following slow speed centrifugation (600 x gravity (G) for 15 min) and six days of further settling were greater than those observed in samples that were centrifuged for 15 min at 30,000 x G.

2. Both high and low speed centrifugation of drilling mud suspensions yielded supernatants with barium concentrations in excess of those expected based on the solubility of $BaSO_4$ in sea water; barium concentrations significantly above background occurred in mixtures (1 ppm mud concentration) of the medium density lignosulfonate muds tested ("Sept. 4" and "Gilson").

   Sea water suspensions (10 mL mud in 990 mL sea water) of the three muds labelled "May 15", "May 29", and "Sept. 4" contained no detectable amounts of cadmium, mercury, nickel, lead, or aluminum. Results from the measurements of trace metals in drilling fluid - sea water mixtures showed that the average concentrations of the detectable elements decreased in the order Ba>Cr>Mn>Zn>Cu (in drilling fluids obtained from EPA, API, and PESA that were labelled: "AN31"," MIBLKA51", "SV76", "PI", "P2", "P3", "P4", "P5", "P6", "P7", "P8", and "Sept. 4".).

3. Early comparisons of drilling mud toxicity data with the total chromium content of the muds revealed no clear correlation relationship. However, metal speciation is an important factor in bioavailability and toxicity. An analysis of chromium speciation showed that the liquid phase of the mud designated SV-76 had the highest concentration of Cr(VI), the most biologically toxic form of chromium. This phase was also one of the most toxic to *Mercenaria mercenaria* larvae.

4. The drilling muds labelled "Gilson", "May 15", "May 29", and "Sept. 4" contained lipophilic fractions that were similar to #2 fuel oil. The "Sept. 4" mud contained approximately 1.15 mg of hydrocarbons per gram of mud that were similar to #2 fuel oil hydrocarbons. Drilling muds that contained organic components similar to #2 fuel oil were more toxic than those that did not.

5. In acute toxicity tests with the copepod *Acartia tonsa*, "Exxon" was the least toxic mud while "May 15" was the most toxic. Toxicity of the muds to *A. tonsa* showed an inverse relationship with the amount of time that the test suspensions were allowed to settle prior to the assay. Filtration of the test suspensions greatly decreased the toxicity of the "May 29" mud but not the "Sept. 4" mud, suggesting that the former contained toxic agents that were associated with the drilling mud particulates while the latter apparently contained dissolved or colloidal toxicants. Lignosulfonates did not by themselves appear to be the principal toxicants to *A. tonsa*. *Centropages typicus* was equally as sensitive as *Acartia tonsa* to "May 15" and "Sept. 4" test suspensions that had settled for 3 days, although *C. typicus* was more sensitive to filtered "Sept. 4" mud and to water soluble components of #2 fuel oil than was *A. tonsa*.

   Test results indicated that juvenile *A. tonsa* were not more sensitive to toxic drilling mud solutions than adults of the same species, and that decreased fecundity occured among adult *A. tonsa* at concentrations of drilling muds which were only slightly below those that caused mortality.

6. A 48 h exposure to as little as 1 mL/L of the "Sept. 4" mud produced a significant mortality of sea scallop larvae (*Placopecten magellanicus*); exposure to 3 mL/L of this mud caused 100% mortality of these larvae. The "Exxon" mud had no measurable effect on survival or shell development of *P. magellanicus* larvae. Shell development was arrested in sea scallop larvae that were exposed for 96 h to: > 0.1 mL/L of the "Sept. 4" mud; 0.3 mL/L of the "May 29" mud; or > 1 mL/L of the "May 15" mud. Arrested shell development appeared to be a sensitive indicator of toxicity in bivalve larvae induced by exposure to drilling muds.

7. In "liquid phase" (settled 72 h prior to use) toxicity tests with larvae of the quahog *Mercenaria mercenaria*, no fertilized eggs developed to the earliest shelled larval stage ("straight-hinge") when exposed for 48 h to concentrations of 500 mL/L or greater of the "Sept 4" mud, while at a concentration of 150 mL/L of this mud 68% of the fertilized eggs developed to shelled larvae).

8. In studies on the toxicity of the drilling muds to crab larvae (*Cancer irroratus*), no significant differences in mortality or number of molts was evident between any of the treatments and the controls, even at mud concentrations that produced abnormal shell development in scallop larvae (*P. magellanicus*). Exposure of crab larvae to the "Sept. 4" mud at a concentration of 100 uL per liter of sea water temporarily inhibited feeding. In general, crab larvae appeared to exhibit the same general toxicity responses as the copepods.

9. Toxicity tests showed that none of the four muds labelled "Exxon", "Gilson", "May 15", and "Sept. 4" exhibited acute toxicity to young flounder (*Pseudopleuronectes americanus*) when 8.7 ml mud was mixed with 1 L sea water, allowed to settle 30 min, decanted and the supernatant mixed with sea water to yield test suspensions of 30, 10, 3, and 1% supernatant. No flounder died during the 48 h test or during a 48 h recovery period. In addition, exposure of *P. americanus* eggs to "Exxon", "Gilson", and "Sept. 4" mud suspensions had no detectable effect on fertilization, although the drilling muds appeared to have an agglutinating effect on the sperm.

10. In additional toxicity tests, the "Sept. 4" and the "May 15" muds were relatively non-toxic to the Atlantic silverside minnow, *Menidia menidia*. All minnows survived when exposed to the undiluted, settled test suspensions of these muds. However, the "May 15" mud was toxic to the mysid shrimp *Neomysis americana* (96 h LC_{50} 0.81 mL/L), and the copepod *Acartia tonsa* (96 h LC_{50} 0.39 mL mud/L).

11. In studies to analyze the effects of drilling muds on the recolonization of defaunated sediments, the presence of drilling mud either layered on top or mixed with reference sediment inhibited recolonization. The presence of drilling mud also affected the distribution of species that successfully recolonized the mud/sediment test phases. In the recolonization studies, *Capitella capitata* was much more numerous in sediments that contained drilling mud.

    In general, data showed that a used PESA drilling mud decreased recolonization when layered (0.4 cm) on top of defaunated reference sediment (3.6 cm), but not when mixed (1:4) with it. The deposition of a new layer of detrital material on top of drilling mud seemed to reduce or reverse these effects; after four to six weeks exposure of defaunated test sediments in the field, the effects were no longer obvious. Greater numbers of animals occurred in field recolonization experiments than in a lab-based, flow-through recolonization set-up. Recolonization studies, although lengthy, were generally found to be an improvement over traditional solid-phase toxicity tests as a method for measuring the impact of contaminated sediment on the benthic environment.

12. Results showed that the methods used for preparing drilling mud test media affect the apparent toxicity of the muds. In "liquid phases" of drilling muds (i.e., mixtures of mud and sea water that were allowed to settle for some period of time) toxicity was generally found to decrease with increased settling time. Filtration further decreased the toxicity of the "May 29" and the "Gilson" muds. Settling for as little as 1 hour reduced the toxicity of the "May 15" mud, while settling was found to yield only slight reductions in the toxicity of the "Sept. 4" mud.

INTRODUCTION

DRILLING FLUID STUDIES AT THE EDGERTON RESEARCH LABORATORY, NEW ENGLAND AQUARIUM

Several studies have been conducted in our laboratory to investigate the chemistry and marine toxicology of a number of drilling fluids, including "Mobile Bay" ("May 15," "May 29," and "Sept. 4"), "Jay Field," "Gilson," "Exxon," and "PESA" ("AN31", "MIBLKA51," "SV76," "P1," "P2," "P3," "P4," "P5," "P6," "P7," and "P8"). A brief discussion of the results of the early studies is appropriate at the start of this report to allow a synthesis of both the chronology and the evolution of our research program on the toxicity and chemical composition of used oil drilling fluids. The terms "drilling fluid" and "drilling mud" are used interchangeably throughout this report, as are the labels ppm (parts per million) and μL/L (microliters per liter). The names of the drilling muds used in this research program are introduced here in quotations to indicate their use as code names for specific specimens provided by EPA, API, or PESA; throughout the remainder of this report, quotations are used only where their omission could lead to confusion.

Studies conducted from the fall of 1979 to the summer of 1980 dealt with: (1) the physical and chemical composition of Mobile Bay, Exxon, and Gilson drilling muds; (2) acute toxicity testing using a number of different marine animal species; (3) fertilization efficiency, egg and larval development of flounder; and (4) benthic recruitment studies (New England Aquarium, 1980).

The results of our trace metal characterization studies indicated that drilling mud suspensions prepared by slow centrifugation (600 x gravity (G); 15 min) to simulate actual oceanic discharges, had higher metal concentrations than samples prepared by high speed centrifugation (30,000 x G; 30 min). In particular, suspensions of medium density mud at a concentration of 1 ppm yielded barium concentrations that were significantly above background. Organic analysis indicated that four of the drilling muds which we tested contained lipophilic fractions that were similar in composition to #2 fuel oil.

Acute toxicity studies using the estuarine copepod, Acartia tonsa, showed that three of the Mobile Bay muds (Sept. 4, May 15 and May 29) produced toxic effects, and that the techniques used in the preparation of mud phases affected their toxicity (i.e., time of settling after mixing; filtered vs. non-filtered; filtration with extraction; and dilute phase preparation method). The May 15 mud was tested under only two treatment conditions, non-settled and settled for 1 hour (h), with respective 96 h LC50 values of 0.03 and 0.39 mL mud/L sea water. LC50 (96 h) values for the May 29 mud ranged from 0.09 to 25.0 mL mud/L with the highest toxicity related to the presence of solids that initially were in the suspension. The 96 h LC50 values for the Sept. 4 mud (0.60 to 1.74 mL/L) indicated a possible effect from dissolved or colloidal species. Further analysis indicated that lignosulfonate-solubilized hydrocarbons could be a possible toxic agent in drilling muds.

Toxicity studies were conducted also on the mysid shrimp, Neomysis americana, and the Atlantic silverside, Menidia menidia. The results of these tests indicated that both the Sept. 4 and May 15 muds were relatively non-toxic to M. menidia, although mortality was observed with the mysid, N. americana, for which a 96 h LC50 value of 0.81 mL mud/L was obtained. In additional tests, juvenile winter flounder, Pseudopleuronectes americanus, showed no acute toxicity following a 96-h exposure to the May 29 drilling mud at 8.7 mL mud/L (mixed 30 min, settled 1 h).

Tests were conducted to measure the effects of drilling muds on the fertilization efficiency of P. americanus. Exposure to the liquid and the suspended solid phases (8.7 mL mud/L) of Exxon, Gilson, and Mobile Bay May 15 muds showed no apparent adverse effects on fertilization efficiency. However, microscopical observations indicated that sperm motility seemed to be affected after exposure to drilling muds at a concentration of 1.0 mL mud/L for all of the muds that were tested. The exposure of fertilized eggs to the liquid phase and the suspended solid phase (1.0 mL mud/L) indicated that apparently normal development occurred through the gastrulation stage. Further testing on egg development was prevented by the ending of the P. americanus spawning season.

Similar fertilization efficiency studies were attempted by using the yellowtail flounder, Limanda ferruginea, and the gray sole, Glyptocephalus cymnoglossus. Species availability during spawning season and the constraints associated with shipboard experiments proved insurmountable for extensive testing with both of these species. Ultimately, further experimentation with gametes and larvae of fish was terminated in favor of the more promising area of invertebrate toxicological assessment.

A field study of recruitment and recolonization was conducted using layered fractions of Exxon drilling muds over natural sediment in a sheltered embayment. Preliminary results, although inconclusive, indicated a suppression of overall population size and a general decrease in species diversity in the treatment with drilling mud, compared to the recolonization and recruitment seen in the control sediment.

Research from the summer of 1980 to the spring of 1981 centered on further investigations of the chemistry and toxicity of spent drilling fluids, including: (1) toxicity testing with two species of copepods; (2) chemical characterization of the test-phase preparations used in toxicity bioassays; (3) bioaccumulation of trace elements in organisms exposed to drilling muds; (4) distribution of trace elements in sediments and water; (5) effects of drilling muds on larval development of sea scallops (Placopecten Magellanicus) and rock crabs (Cancer irroratus); and (6) effects of drilling muds on colonization by benthic organisms (New England Aquarium, 1981).

Acute toxicity tests were continued by using Acartia tonsa and a second copepod species, Centropages typicus, which is common on Georges Bank. As found in the earlier studies, the method of test phase preparation affected the observed toxicity. Among the phases prepared from the May 15 and the May 29 Mobile Bay mud, toxicity to A. tonsa decreased with increased mud settling time. In addition, the Sept. 4 Mobile Bay mud displayed greater toxicity when the individual concentrations in the test series were prepared separately, as opposed to the preparation of the same concentration series by sequentially diluting a primary stock suspension. The other species of copepod tested (C. typicus) was as sensitive as A. tonsa (0.49 - 1.5 mL mud/L = 96 h LC50 value) to phases of the Sept. 4 and May 15 mud suspensions which were allowed to settle for three days.

Results of fecundity and juvenile exposure studies on *A. tonsa* indicate that decreased fecundity occured among adults at exposure concentrations only just below those which caused mortality, and that juveniles exhibited responses to drilling muds that were similar to adults of the same species.

A study was initiated to determine which chemicals may contribute to the toxicity of some drilling muds. Test phases used in the toxicity tests were analyzed for both trace metal and organic components. Chromium was targeted as a possible toxic component because high concentrations of this metal were found in sea water suspensions of Mobile Bay drilling mud. Early results from toxicity tests with both of the copepod species indicated that chromium did not appear to play a dominant role in determining drilling mud toxicity. However, metal speciation is an important factor in bioavailability and toxicity, and further investigation of chromium speciation showed that the liquid phase of the mud labelled SV-76 had the highest concentration of Cr(VI), the most biologically toxic form of chromium. This phase was also one of the most toxic to *Mercenaria mercenaria* larvae. Further analyses are needed to determine the speciation, and metal-binding properties of drilling mud components. The measurement of sea water-soluble and acid-soluble elements in the ten drilling muds that were tested showed detectable concentrations above background for barium and chromium, as well as the release of aluminum, iron and manganese. Aluminum, iron and manganese occurred at concentrations likely to be minimal in impact considering dispersion and dilution factors. Organic analysis indicated that all test phases contain tributyl phosphate, alkyl-substituted catechols and polycyclic aromatic hydrocarbons. In addition, the test phase produced by the Sept. 4 mud contained a naphthalene-based ketone.

A study of trace element concentrations was conducted on samples of the drilling mud test phases taken during the course of 96-h toxicity tests conducted at the National Marine Fisheries Service, Sandy Hook laboratory with PESA drilling muds. Results of these studies indicated that, although the concentrations of eight trace elements were very low in the sea water test media, the settled drilling mud continued to release metals when re-mixed with sea water.

Analyses were conducted to measure the bioaccumulation of trace metals by red-winged oysters (species unknown), scallops (Placopecten magellanicus), and sea urchin (species unknown) spines exposed to Jay Field drilling mud suspensions. In the soft tissue of the scallop and oyster, only barium concentrations increased with increased exposure to drilling muds in the 10 ppm and 100 ppm range. Following even one week of depuration in clean water, animals exposed previously to 100 ppm drilling muds exhibited elevated concentrations of Ba compared to background. Analysis of oyster shells showed an increase in zinc over background in animals exposed to 10 ppm and 100 ppm drilling mud. For both the oyster shells and the sea urchin spines, barium concentrations were lower in the controls than in animals exposed to 10 and 100 ppm drilling fluid, but were higher in the controls than in animals exposed to 1 ppm drilling fluid. The resulting data gave no clear explanation for this unusual phenomenon.

A number of toxicity studies were conducted by using the larvae of the sea scallop, Placopecten magellanicus. The results indicated that with the Sept. 4 mud as little as 1 mL mud per L produced significant mortality among larvae exposed for 48 hours. Arrested shell development was observed after 12 hours among the test populations exposed to all concentrations of Sept. 4 mud and to the highest concentrations of May 15 and May 29 muds. A 96 h exposure to either 1 mL/L of the May 15 mud or 0.3 mL/L of the May 29 mud significantly inhibited shell formation. The Sept. 4 mud was considerably more toxic; it almost completely arrested shell formation at 0.1 mL/L.

Test populations held for 6 days in clean sea water following a 96 h exposure to Sept. 4 mud exhibited an inability to recover from arrested shell formation. None of the organisms exposed to 3 mL/L or 10 mL/L filtered phases of this mud survived. Organisms exposed to liquid phases of the two May muds were also unable to recover over a 6-day period in clean sea water following exposure to the Sept. 4 mud.

The results of the 96 h exposures indicate that the Gilson mud was as toxic to the larvae as the May muds from Mobile Bay, although it was less toxic than the Sept. 4 mud. The filtered test phase of the Exxon mud had no measurable effect on survival or on shell development.

Arrested shell formation in bivalve larvae appears to be a sensitive indicator of stress induced by drilling muds. The results of 96 h tests in which a variety of marine fauna were exposed to filtered mud suspensions indicated that copepods were the most sensitive of the species tested. LC50 values (96 h) for _Acartia tonsa_ exposed to filtered phases of the Sept. 4 dilling mud were between 1 and 2 mL/L. However, considering sub-lethal effects, a 96 h exposure to 0.1 mL/L arrested shell development in sea scallop larvae.

Twenty-day larval development experiments were conducted with the brachyuran crab, Cancer irroratus. The larvae were exposed for 24, 48, or 72 hrs to 5, 10, and 100 uL/L concentrations of Sept. 4 dilling mud. Both mortality and the number of molts were recorded over the 20-day test period. Results indicated no significant differences between any of the treatments and the control for both mortality and number of molts, even at concentrations that produced abnormal shell development in the sea scallop.

Feeding experiments with the crab larvae indicated that exposure to a 100 uL/L test phase of Sept. 4 drilling mud can temporarily **inhibit their feeding.** However, the effect did not persist once the exposure ended, and there did not appear to be any long-term effect on crab larval growth.

More concentrated suspensions of the Sept. 4 drilling mud were acutely toxic to 5-day-old crab larvae. The 96 h LC50 value was 1.02 mL of settled, decanted liquid phase per liter of sea water. This result suggests that the larvae were as sensitive to toxic drilling muds as the copepods that we have tested.

The recruitment and recolonization studies centered on the testing of a variety of laboratory designs to develop a functional year-round experimental system. Two systems that were evaluated included a longitudinal trough design and a system utilizing a circular tank. After several modifications, the circular tank design provided a reliable laboratory system with a uniform water circulation pattern. In addition, a field-based system was designed to augment the laboratory studies with several objectives in mind. The first objective was to document any effects of drilling mud on either the rate of colonization of defaunated sediment or the species composition of the recolonizing population. Secondly, we intended to determine what time period was most critical during the recolonization process. Finally, we compared results of concurrent laboratory- and field-based experiments. For a more detailed description of these studies, refer to New England Aquarium Progress Report #2 (1981), and the results of these studies which are contained in Part IV of this report.

Research conducted between May 1981 and February 1982 dealt with two major areas of interest. The first was the construction and development of a mariculture facility to allow year-round larval production of the hard clam, *Mercenaria mercenaria*, for drilling mud tests. The second dealt with the results of both laboratory and field studies on the effects of drilling fluids on benthic recolonization (New England Aquarium, 1982).

The operation of the mariculture system over a four-month period proved that the system is capable of maintaining and conditioning adult *M. mercenaria* for year-round spawning. Procedures developed for liquid phase testing of larval *M. mercenaria* represented a feasible standard protocol. Preliminary results showed that when 1-h-old larvae are exposed for 48 h to the liquid phase of the Mobile Bay Sept. 4 mud (0.5, 1.5, 5.0 and 15 ppt), failed to form shells, while control animals developed normal shells. There was no significant difference in shell formation between fed and unfed animals in these treatments. Observations were also made on the effects of these liquid phases on larval embryogenesis. Growth inhibition was found to be directly related to concentration.

Results of the drilling mud recolonization studies are contained in the Part IV of this report. As a method for measuring the impact of contaminated sediment on the benthic environment, the recruitment studies were generally found to be an improvement over traditional solid phase toxicity tests that have been used for assessing the impact of dredged materials. However, the time required for both testing and data processing in recruitment studies is too lengthy for the efficient evaluation of whole drilling muds.

The following report outlines studies conducted by the Edgerton Research Laboratory concerning the toxicity and chemical composition of spent PESA drilling fluids.

I. EFFECTS OF USED DRILLING FLUIDS ON THE EMBRYONIC DEVELOPMENT OF THE HARD CLAM, *Mercenaria mercenaria* (L.)

1.1 Background

Drilling fluids, or drilling muds, are vital to the offshore exploration for oil and gas because they fulfill the requirements for drill bit lubrication, bore hole stabilization to prevent cave-ins and blow-outs, and for removing rock chips (cuttings) from the cutting surface of the drill bit. These and other tasks are performed by circulation of the barite-rich (approximately 94 percent $BaSO_4$) mud down the bore hole to the drill bit from which cuttings are carried to the surface for removal and subsequent discharge into the ocean. The composition of a discharged drilling mud is altered from its original state by the drilling conditions (geological formation, temperature, and pressure) (IMCO Services, 1978; Perricone, 1980) and thus the complex mixture of organics (Strosher, 1980) and trace metals (Liss *et al*., 1980) in the mud can vary. The composition of the mud is changed in response to the drilling conditions and, consequently, the subsequent effects of discharged muds on marine organisms may also change over the duration of a drilling operation. This potential for changes in mud toxicity as a function of drilling conditions was recently reported in laboratory toxicity tests by Tornberg *et al*. (1980) and Conklin *et al*. (1983) who showed that mud toxicity increased with drilling depth.

The toxicity of used drilling muds to marine fauna is well established (Sprague and Logan, 1979; Houghton, Beyer, and Thielk, 1980; Thornberg *et al*., 1980; Neff *et al*., 1980; Thompson and Bright, 1980; Conklin *et al*., 1980; Carr, Reitsema, and Neff, 1980; Gerber *et* al., 1980; Gerber et al., 1980, Neff et al., 1980; Carr et al., 1980; Crawford and Gates, 1981; Chaffee and Spies, 1982). The discharge of cuttings and muds into the pelagic zone and their subsequent impact on the early life stages of the seasonal plankton has only begun to be addressed. Most studies on drilling mud toxicity have concentrated on adult animals from the benthic community. Although it has been demonstrated that adults can often survive high concentrations of drilling fluids, the ultimate survival of an impacted population in succeeding generations depends to a great degree on the tolerance of the planktonic early life stages. Although juveniles of a few fish species have been shown to exhibit greater sensitivity to metals as they age (Chapman, 1978; Blaxter, 1977), the generally greater sensitivity of early life stages is well documented in both the plant and animal kingdom for a number of environmental and anthropogenic stresses (Odum, 1962; Portmann, 1970; Buikema and Benfield, 1979; Neff et al., 1980; Carr et al., 1980).

This study was initiated in response to concern over the impact of offshore drilling activities on commercially important bivalve molluscs and the lack of information concerning the effects of drilling muds on molluscan larvae. A diverse collection of used drilling muds was used to evaluate the sensitivity of the hard clam, Mercenaria mercenaria, fertilized eggs (1-h post fertilization). M. mercenaria was chosen because of its wide climatic range in the coastal zone of the United States and because adult brood stock can be held for year-round spawning and subsequent toxicological testing without gonad resorption (Loosanoff and Davis, 1950). Dilute sea water suspensions were used in all the tests with the goal of approximating "real-world" test conditions.

1.2 Materials and Methods

The natural sea water used for maintaining marine tanks at the New England Aquarium was used to culture *M. mercenaria* and its algal food. The water for all tests was filtered through a series of cotton filter cartridges to remove particles greater than 0.45 um. The salinity of the sea water was 30-32$^{0}/_{00}$; pH was 7.9 ± 0.2, and dissolved oxygen content was at least 85% of saturation throughout the analysis.

1.2.1 Conditioning and Holding of Brood Stock.

Adult *M. mercenaria* (approximately 50-60 g) were collected from the mouth of the Marstons Mills River in Cotuit (Cape Cod), Massachusetts, at various times of the year. The clams were transported immediately to the lab in a styrofoam cooler at ambient temperature and then slowly acclimated to the conditioning (15°C) or holding (11°C) tank temperature.

During the conditioning period (minimum of 6-8 weeks), no more than 80 (50-60 g size) brood stock animals were maintained at 15.0 ± 1.0°C in two deep trays (76x91x25 cm) containing 8-10 cm of sand from the area in which the animals were collected. Before the clams were introduced into the conditioning tray, the date was marked on the shell with an indelible ink marker, and the clams were placed on the sand and allowed to dig into the substrate. The conditioning trays received approximately 200 L/day of cultured algae and approximately 1000 L/day of filtered (1 um) sea water, amounting to approximately 6-8 water changes/day. Depending on the cell densities of the algal cultures, the adult animal conditioning trays contained from 0.4 to 1.0 x 10^5 algal cells/mL at all times.

Feeding was provided by the continuous harvest of 360 L, fiberglass-tube cultures (Solar storage tube, Kalwall Corporation, Manchester, N.H.) of mixed or unialgal cultures of the diatom _Thalassiosira_ _pseudonana_ (3H strain) and the flagellated chrysophyte _Isochrysis_ _galbana_ (Tahitian strain). Algal harvesting was carried out with a small diaphragm pump. The mass culturing of microalgae was initiated after the sea water was chlorinated overnight (60 ml chlorine/360 L), and then dechlorinated with sodium thiosulfate (10 g/360 L). Eight to twelve one-liter algal stock cultures were added after the water was supplemented with Guillard's F/2 nutrient supplement (Guillard, 1974).

Newly collected animals which could not be accommodated in the conditioning trays were acclimated to the 11 ± 1.0°C holding temperature and held until they were needed for conditioning. These animals either received continuous feeding from the overflow of the conditioning trays, which contained an excess of algae (greater than 4×10^4 cells/mL), or they were fed 15-20 L batch quantities from the algal tube cultures daily. This cold-water holding system consisted of two 350-L insulated trays maintaining 200 adult clams. The sea water flowed into the holding trays at a minimum rate of one turnover per day.

1.2.2 Spawning and Rearing.

Conditioned brood stock animals were spawned by the methods described by Loosanoff and Davis (1963). Fertilization was achieved when the pooled sperm and eggs were mixed and allowed to remain in contact for 1 h. Depending on the sperm concentration, approximately 6 mL of sperm were mixed with 1 liter of egg suspension to yield between 10^5 and 10^7 sperm/mL. Lower sperm concentrations can result in incomplete fertilization, while higher levels can yield larval deformities, probably from polyspermy (Culliney *et al.*, 1975). After a 1 h fertilization period, the fertilized eggs were sieved onto a 63 um Nitex screen to remove sperm, and were resuspended in a one liter beaker containing 0.45 um filtered sea water. The egg suspension was mixed gently with a perforated Teflon plunger to yield a uniform suspension before a sample was taken with a micropipette for the quantification of eggs at 25x under a dissecting microscope. Eggs were counted in triplicate 100 uL aliquots and the mean was multiplied by 10 to give the number of eggs per mL. To reduce variability in sampling fertilized eggs, two separate aliquots of the uniformly mixed egg suspension were inoculated into each tube to yield a final density of 10-15 embryos/mL.

Test vessels consisted of round-bottom, 60 mL Pyrex test tubes containing 50 mL of the drilling mud test phases. The test vessels were held at 27°C ±1°C in a constant temperature bath. Each test vessel was aerated for 60 min prior to inoculation with fertilized eggs to increase the dissolved oxygen concentration.

A specially designed water bath was made to contain the test vessels at 27°C ±1°C for the 48 h incubation period (Fig. 1).

This unit consisted of the following components: (1) a large styrofoam cooler (75x35 cm) with holes (2.5 cm diameter) bored into the cover to hold the test vessels; (2) a Neslab-Endocol constant temperature circulator to provide a flow of water through the cooler for bath temperature maintenance; and (3) an air manifold system to ensure uniform bubbling within each test tube. The air was filtered through an oil separator to remove any residual oil and piped by a manifold through 0.22 mm (ID) teflon tubing to each test vessel.

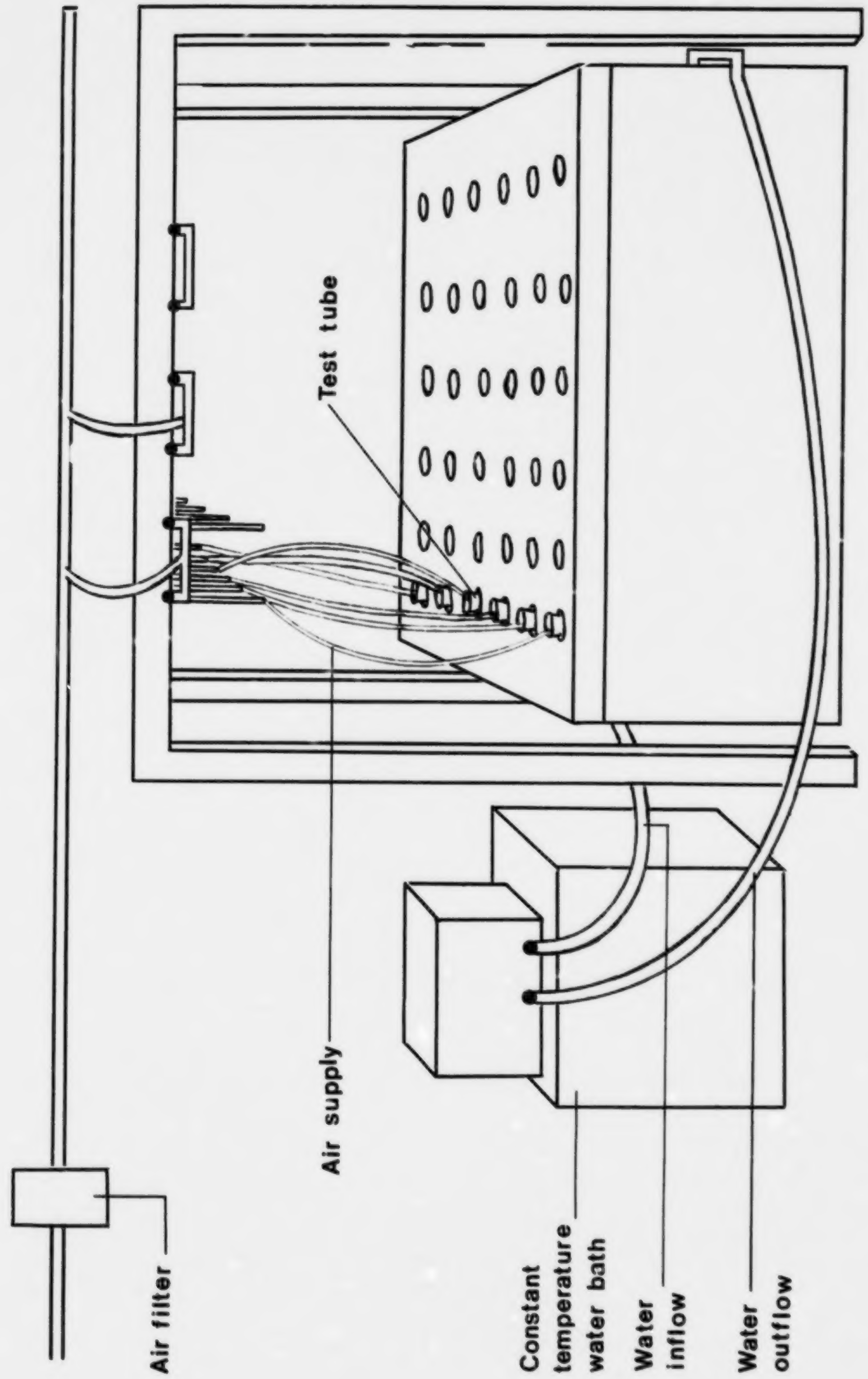
1.2.3 Experimental Procedure.

Drilling muds were provided by the Petroleum Equipment Suppliers Association (PESA) and distributed by the Environmental Protection Agency, Gulf Breeze, Florida. These muds were collected from various drilling sites, identified by an EPA code and categorized according to mud type.

Test phases were prepared with the intent that they would closely approximate the actual composition of drilling mud suspensions released into the ocean. Dilute drilling mud/sea water mixtures were prepared by the addition of from 0.15 to 3 mL of used drilling mud into a liter of 0.45 um filtered sea water. Two phases were prepared from each drilling mud to test the toxicity of both the water soluble components alone (liquid phase), and the suspended solids plus water soluble components (suspended-solids phase). Two-liter glass bottles were washed first with acetone and then with 6N HCl to remove adsorbed organics and metals, and were then rinsed three times with distilled water. The liquid and suspended-solids test phases were prepared by adding a volume of the drilling mud to two liters of 0.45 um filtered sea water. These dilute suspensions

FIGURE 1

UNIT FOR TESTING EFFECTS OF DRILLING FLUIDS ON CLAM EMBRYOS


Air filter
Air supply
Test tube
Constant temperature water bath
Water inflow
Water outflow

of drilling fluids were stirred for 30 min with a Teflon-coated magnetic stirrer and allowed to settle at room temperature for either 1 h, to produce a suspended-solids phase, or for 72 h, to produce a liquid phase. After the appropriate settling period, the test phases were collected by siphoning for use in the bioassay procedure. Particles with the density of clay and dimensions of 0.5 um or greater in diameter settled out of the test phases after 72 h. This procedure was preferred over membrane filtration because it achieved the same results without the problems of degassing and adsorption of toxicants to the membrane filters. In addition, the settling method more closely resembles conditions found at sites where drilling muds are released.

In order to allow the testing of several muds at different times of the year, the use of several different adult brood animals was required for spawning larvae, and this necessitated the inclusion of a standard toxic control mud to allow comparison between tests. For this purpose we chose a used saltwater lignosulfonate mud identified as Mobile Bay - Sept. 4, collected off the coast of Alabama. in 1979. Prior to testing, each test vessel was acid washed (6N HCl), rinsed with distilled water, and placed in a 550°C oven for 60 min.

Drilling fluid concentrations were tested in triplicate and each test vessel was placed randomly into the larval bioassay test apparatus along with triplicate controls. The eggs were added to the liquid phase within one hour after fertilization. Dissolved oxygen, pH, and salinity were measured at the beginning and end of each test. Temperature was monitored throughout each 48-h test period.

The number of D-stage, shelled prodissoconch-I (straight-hinge, or D-stage) larvae that developed with normal shells in the control containers was compared to the number of normal-shelled, D-stage larvae that developed from the embryos in the test containers to develop an index of toxicity in these tests.

After the 48-h static testing period, 1 mL of 10% phosphate-buffered formalin and 2.5 mL of 0.5 % Rose Bengal (both in 0.45 um filtered sea water) were added to each test vessel and the preserved samples were stored in a refrigerator at $4^{0}C \pm 2^{0}C$. The contents of each vessel were filtered through a 25-um Nitex screen to retain the larvae. The screen was washed three times and all organisms were transferred to a Petri dish. A binocular dissecting microscope was used to count the larvae.

1.2.4 Statistical Analysis.

The results of these tests were expressed as 48-h EC50 values related to the proportion of the test population which failed to develop into straight-hinge, prodissochonch-I larvae (D-stage). These values and their 95% confidence limits were calculated by using probit analysis (Finney, 1971).

1.3 Results

1.3.1 Year-round Toxicological Testing.

Year-round toxicological testing of used drilling muds continued throughout the 15-month analysis period. A temperature of $15 \pm 1.0^{0}C$ was optimal for the year-round conditioning of Mercenaria

mercenaria brood stock. Higher temperatures caused the accidental spawning of ripe broodstock and lower temperatures reduced feeding activity and lengthened the conditioning period.

Our objective in the mass-scale culturing of microalgae was to provide the clam brood stock with a reliable supply of nutritious food which was free of toxicants and pathogens. This was easily accomplished by using 360 L fiberglass tube cultures of Thalassiosira pseudonana (3H strain) and/or Isochrysis galbana (Tahitian strain) at a density of 1 to 4×10^6 cells/mL. Sufficient food was supplied by one 360-L culture to sustain the nutritional requirements of the brood stock animals in the flow-through conditioning trays for 2-3 days. The algal mass cultures were pumped continuously into the conditioning trays to yield a constant supply of algae (approx. 10^4 cells/mL) to the brood animals. Three mass-culture tubes were ample for culturing these algae as food for the year-round conditioning of brood stock. The cultures attained a harvest density of $1\text{-}4 \times 10^6$ cells/mL approximately 4-5 days after it received its F/2 nutrient supplement (Guillard, 1974) and algal inoculum.

1.3.2 One-hour Fertilized Egg Toxicity Test.

A summary of the data from 12 used drilling muds is presented in Tables I-A and I-B, and Figures 2 and 3. The toxicity is presented for both liquid (72 h settled) and suspended-solid phases of for each mud . The suspended-solids phases of muds P1, P2, and P3 (which also includes the water soluble fraction) were found to be more toxic than the water-soluble fractions alone. The remaining muds did not show an appreciable difference in toxicity between the two mud phases.

TABLE I-A: 48 HOUR DRILLING FLUID TOXICITY TEST: SUSPENDED SOLIDS PHASE

Continuous exposure (48 h) of 1 hour old, fertilized eggs of *Mercenaria mercenaria* to the suspended solids phases of various drilling fluids showing percent of each test group (n = 625 ± 125 eggs) that developed into normal, "D" stage larvae.

Drilling Fluid	Test Date	EC50	Confidence Limits		Control
			LCL*	UCL*	% "D" Stage
AN31	5/20/82	1,771 ppm**	1,710	1,831	93
MIBLKA51	1/26/82	>3,000 ppm	--	--	95
SV76	5/20/82	117 ppm	115	119	93
P-1	9/20/82	122 ppm	89	151	99
P-2	9/20/82	156 ppm	149	162	99
P-3	9/20/82	64 ppm	32	96	99
P-4	9/20/82	347 ppm	330	364	99
P-5	9/20/82	382 ppm	370	395	99
P-6	11/22/82	>3,000 ppm	--	--	93
P-7	11/22/82	2,779 ppm	2,667	2,899	93
P-8	11/22/82	212 ppm	200	223	93
Sept. 4 (Mobile Bay)	5/20/82	125 ppm	120	130	93
	9/20/82	97 ppm	94	101	99
	11/22/82	119 ppm	111	128	93

* LCL = lower confidence limit; * UCL = upper confidence limit; (95% confidence limits

** Vol/Vol mixture of a 1 hour-settled drilling mud suspension and 0.45 um-filtered natural sea water to yield the indicated concentration of drilling mud suspended solids phase in sea water.

TABLE I-B: 48 HOUR DRILLING FLUID TOXICITY TEST: LIQUID PHASE

Continuous exposure (48 h) of 1 hour old, fertilized eggs of *Mercenaria mercenaria* to the liquid phase of various drilling fluids showing percent of each test group (n = 625 ± 125 eggs) that develop into normal, "D" stage larvae.

LIQUID PHASE

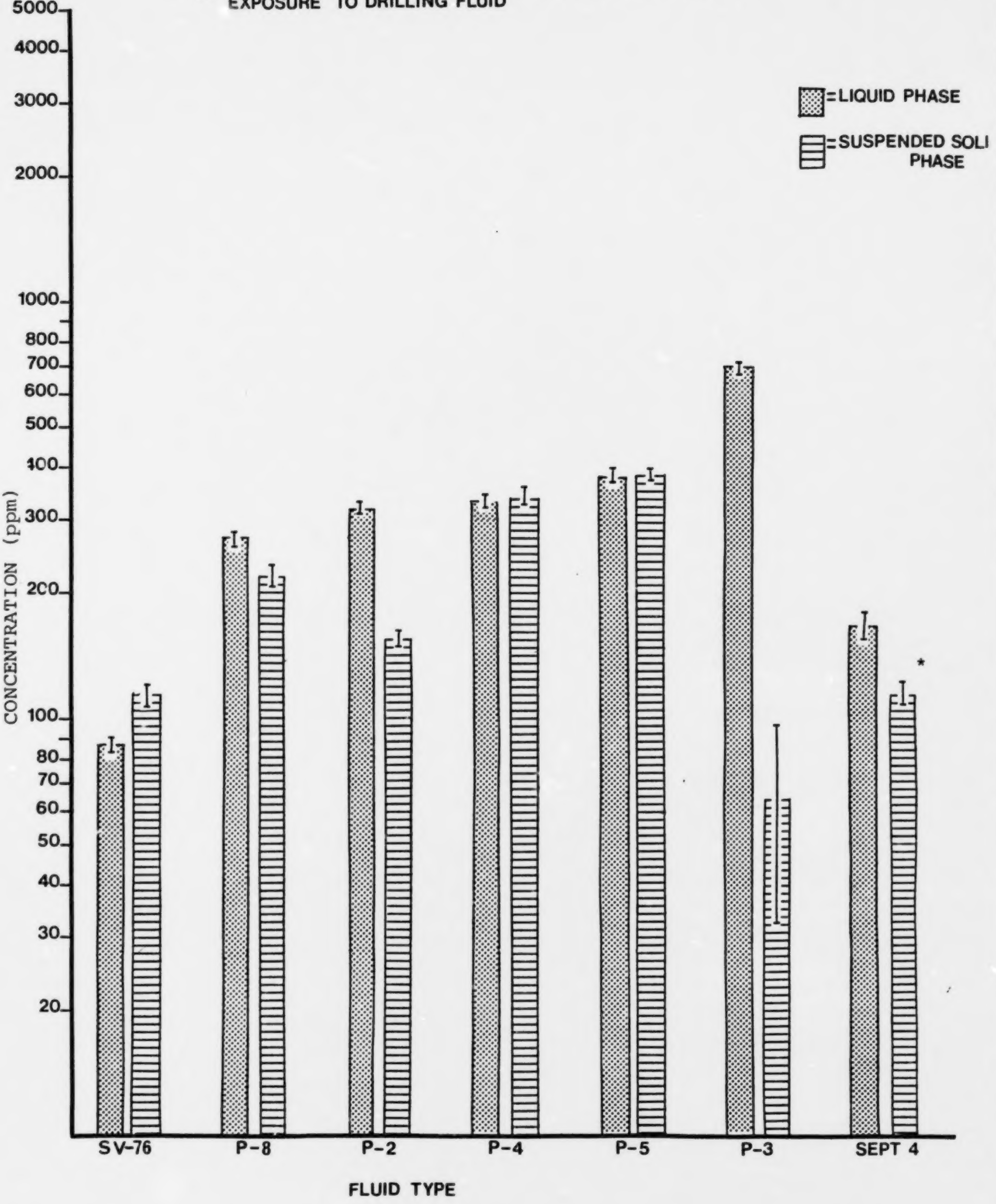
Drilling Fluid	Test Date	EC50	LCL*	UCL*	Control % "D" Stage
AN31	3/15/82	2,427 ppm**	2,390	2,463	88
MIBLKA51	1/26/82	>3,000 ppm	--	--	95
SV76	3/15/82	85 ppm	81	88	88
P-1	6/14/82	712 ppm	690	734	97
P-2	6/14/82	318 ppm	308	328	97
P-3	7/26/82	683 ppm	665	702	98
P-4	7/26/82	334 ppm	324	345	98
P-5	7/26/82	385 ppm	371	399	98
P-6	8/16/82	>3,000 ppm	--	--	97
P-7	8/16/82	>3,000 ppm	--	--	97
P-8	11/22/82	269 ppm	257	280	93
Sept. 4 (Mobile Bay)	3/15/82	134 ppm	126	141	88
	6/14/82	112 ppm	91	122	97
	7/26/82	176 ppm	168	193	98
	8/16/82	187 ppm	170	212	97

*LCL = lower confidence limit; UCL = upper confidence limit; (95% confidence limits)

** Vol/Vol mixture of a 72 hour-settled drilling mud suspension and 0.45 um-filtered natural sea water to yield the indicated concentration of drilling mud "liquid phase" in sea water.

FIGURE 2

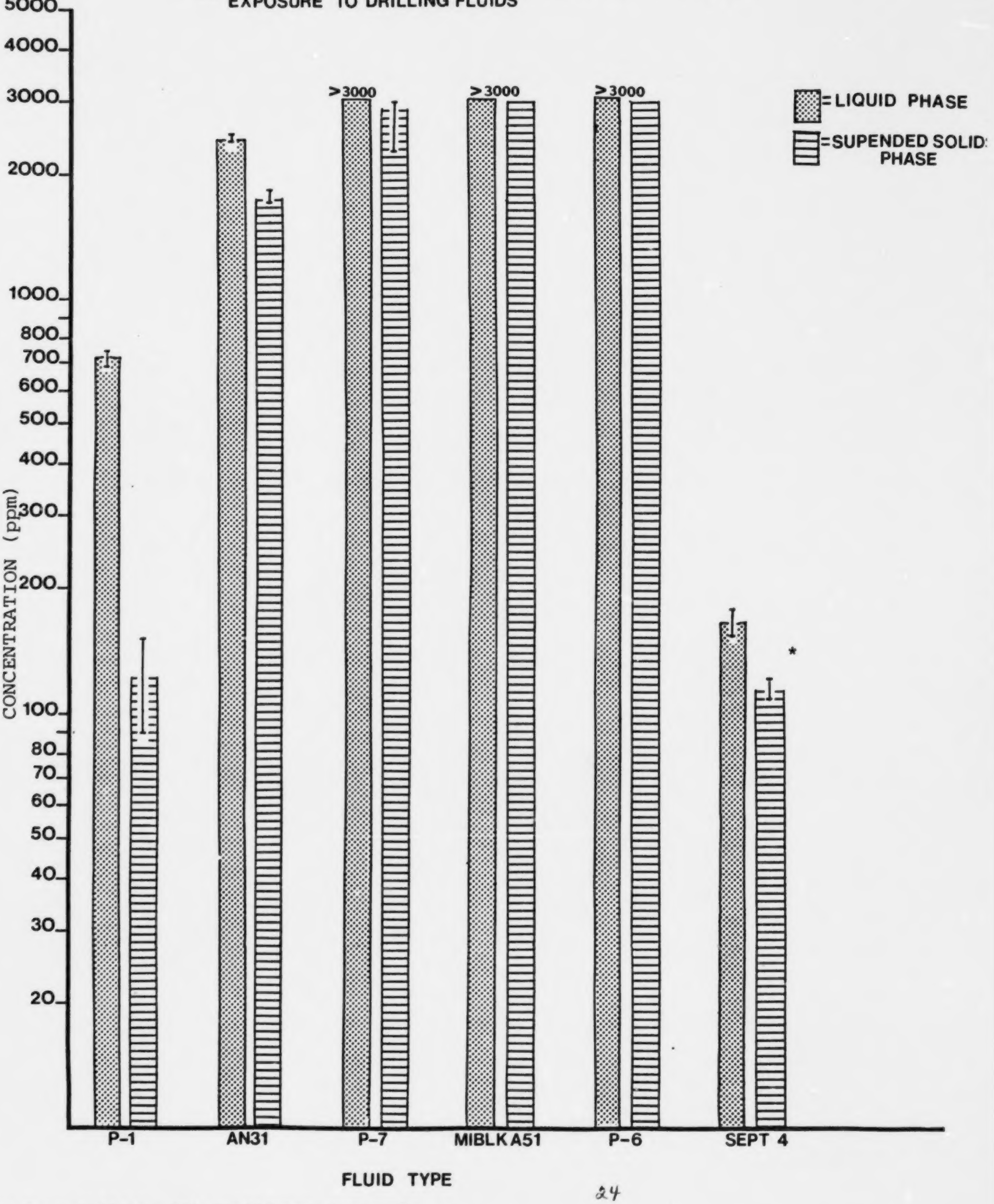
EC_{50} VALUES FOR M. MERCENARIA LARVAE AFTER A 48 HOUR EXPOSURE TO DRILLING FLUID



* Bars indicate 95% Confidence Intervals.

FIGURE 3

EC_{50} VALUES FOR M. MERCENARIA LARVAE AFTER A 48 HOUR EXPOSURE TO DRILLING FLUIDS



* Bars indicate 95% Confidence Intervals.

The range of the 48 h EC50's for the liquid phase of 8 of the 12 muds was from 85-712 ppm and the range of EC50's for the suspended solids phase of these muds was from 64-382 ppm. The EC50's for the remaining muds exceeded 2,000 ppm (v/v mixture of whole mud/sea water prior to settling). More than 88 percent of the control animals achieved D-stage in all of the tests in this study.

1.4 Discussion

The results of these tests showed that fertilized eggs of *M. mercenaria* were very sensitive to both the liquid and the suspended-solids phases of a diverse assortment of used drilling muds. The mud types (Table II) included a "lime" mud (P3), a sea water potassium polymer mud (P8), a low solids non-dispersed mud (P6) and various chrome lignosulfonate types (AN31, MIBLK, Mobile Bay - Sept. 4, SV76, P1, P2, P4, P5, and P7).

The wide range of 48 h EC50 values (64-3000 ppm) indicates large variations in the chemical composition of used drilling muds. As was found by other investigators who also employed early life stages for testing (Crawford and Gates, 1981; Chaffee and Spies, 1982), the 1-h fertilized eggs of *M. mercenaria* showed the lowest 48-h EC50 values when exposed to drilling muds. EC50 values for most muds were in the 64-800 ppm range. The sensitivity of molluscan early-life stages has also been demonstrated for a number of other toxicants (Calabrese, 1972; Calabrese *et al*., 1973; Calabrese and Nelson, 1974; MacInness and Calabrese, 1979; Coglianese and Martin, 1981; Watling, 1982).

TABLE II. CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE USED DRILLING FLUIDS EMPLOYED IN THE *Mercenaria mercenaria* LARVAL TOXICITY TESTS

Drilling Fluid Code	Mud Type	Depth of Well (m)	Density	% Water
AN31	Sea water lignosulfonate	3576	1.50	49.9
MIBLKA51	Sea water lignosulfonate	2277	1.26	68.5
SV76	Sea water lignosulfonate	NI	2.17	27.3
Mobile Bay (9/4/79)	Sea water lignosulfonate	6236	1.55	54.2
P1	Lightly-treated lignosulfonate	4311	1.93	33.8
P2	Fresh water lignosulfonate	4153	2.02	30.0
P3	Lime mud	5241	2.19	26.8
P4	Fresh water lignosulfonate	3645	1.93	33.4
P5	Fresh water/sea water lignosulfonate	3945	2.20	26.3
P6	Low-solids, nondispersed mud	NI	1.22	75.1
P7	Lightly-treated lignosulfonate	3747	1.37	57.0
P8	Heavily-treated lignosulfonate	3760	2.17	27.3

NI= no information available

Other investigations reporting on sensitive toxicity tests include those of Crawford and Gates (1981) on unfertilized sand dollar (Echinorachnius parma) eggs and Conklin et al. (1980, 1983) on grass shrimp (Paleomonetes pugio) molting stages. Crawford and Gates (1981) exposed unfertilized sand dollar eggs for a 15 min pulse exposure to drilling muds and found that fertilization was affected at concentrations between 100 ppm (88 percent fertilization) and 1,000 ppm (6 percent fertilization). Conklin et al. (1980) identified the molting of grass shrimp as a sensitive stage for use in toxicity tests since they showed a 96-h LC50 value range from 363 to 739 ppm for 5 drilling muds.

The Mobile Bay (Sept. 4) mud was used as the standard toxic control throughout the 15 month testing period. It was intended to serve as a reference between groups of larvae that were spawned at different times. During this study, the toxicity of this reference mud (designated "toxic control" or "toxic reference mud") was 150 ± 37 ppm for the liquid phase and 111 ± 14 ppm for the suspended phase. This indicated good reproducibility between tests and spawn groups. These results suggest that the response of the different test populations was sufficiently uniform to allow direct comparison of results from tests performed at different times.

Our toxic reference mud (Mobile Bay - Sept. 4) was also used by Conklin et al. (1983) who called it mud XVII for their 96-h test with Palaemonetes pugio. Our analyses with this mud verified the extreme sensitivity of the M. mercenaria fertilized egg test. A comparison of the sensitivity of these two organisms to this mud shows that the 150 ppm 48 h EC50 for M. mercenaria fertilized eggs was considerably lower than the 740 ppm 96 h LC50 seen in tests of effects on Palaemonentes pugio molting, especially when considering that the M. mercenaria EC50 is for a 48 h test as opposed to the 96 h P. pugio test. A similar test was performed with this same Mobile Bay mud in our lab on the fertilized eggs of the sea scallop (Placopecten magellanicus) (D. Wayne, New England Aquarium, unpublished data). In this study, the Mobile Bay mud elicited toxicity to sea scallop eggs and also inhibited development at a low concentration (100 ppm) in a 48-h EC50 bioassay. Therefore, fertilized eggs of M. mercenaria and Placopecten magellanicus seemed to be equally sensitive to the same toxic mud. The developing embryos and larvae of both mollusc species appeared to be more sensitive to the muds tested in this study than molting grass shrimp.

Davis (1960) and Davis and Hidu (1969) identified the importance of turbidity to the survival of M. mercenaria eggs and larvae. Eggs exposed to silt were found to have an approximate EC50 value of 1.5-2.0 g/L while eggs exposed to kaolin clay had an EC50 of approximately 0.5 g/L (Davis and Hidu, 1969). Drilling muds contain high concentrations of clay and barium sulfate particles (Perricone, 1980) and the drilling muds used in our studies contained between 0 and 55.6% clay, and (for those that had a known barite content), between 75 and 87% barite; at the 0.02-0.855 g/L concentrations of suspended solids typically found in the plume of an oil well discharging approximately 1,000 bbl/hour of used drilling fluid (Ayers et al., 1980), it is quite likely that fertilized eggs could be severely impacted by turbidity effects alone. However, the maximum drilling mud concentrations (3 mL mud per L of sea water) used in our test phases were relatively low by comparison, and it is unlikely that turbidity effects could have contributed to toxicity in any of the suspended-solids phases that were tested in this study.

The 72 h settling time used to produce the liquid phase more closely approximated natural environmental conditions than previous methods which utilized 0.45 um membrane filtration (Neff *et al.*, 1980). Membrane filtration probably removes metals (Truitt and Weber, 1979) and organic components by adsorption (Bates *et al.*, 1983). However, the 72 h (settled) liquid phase of the drilling fluid SV76 was found to contain less water-soluble fuel oil components than the newly prepared suspended-solids phase of this mud which was centrifuged at 1,000 X G for 3 min (Table X, section III). The diminished water-soluble fuel oil content of the 72 h (settled) liquid phase could be attributed to the lengthy incubation period which may have permitted microbiological decomposition of some organic components. The bactericidal ingredients in drilling muds at the 15-3,000 ppm testing concentrations (v/v, whole mud:sea water) may not have been sufficient to prevent biodegradation during the tests on diluted muds . In addition, more volatile components could have been driven off when the test phases were vigorously aerated before inoculation with the fertilized eggs.

The suspended-solids phases contained the largest quantitites of fuel oil-like substances, and these were the most toxic phases for 8 of the 12 muds. However, there was no direct correlation between toxicity and the concentration of the fuel oil-like components of the drilling muds. Yet, studies on the toxicity of drilling muds by

Conklin et al. (1983) on shrimp and by Miller et al. (1980) on vascular land plants identified diesel fuel as the single most toxic drilling mud component when it is present. Until similar standard experiments can be performed, it will be difficult to separate the effects of biodegradation and adsorption on drilling mud toxicity.

Consideration of the high surface area of the mud particulates that is available for adsorption suggests that mud toxicity may be a function of the particulate surface area. Thus, in the presence of a given concentration of a toxic hydrocarbon, a high-particulate, high-surface-area mud, may be less toxic than a mud with less surface area for toxicant adsorption.

Trace metal analyses (Tables III, VII) identified barium and chromium as the most common metal components in the 12 drilling mud samples. In agreement with Conklin et al. (1983), the toxicity of these muds showed no correlation with the content of chromium. Barium is commonly employed as a weighting agent and has been shown to be biologically inert (Cabrera, 1971; George, 1975). While chromium as Cr(VI) is generally known to be the most toxic species of this metal (Mearns et al., 1976), drilling-mud chromium usually occurs as Cr(III) in the form of chromium lignosulfonate chelates or as insoluble chromium hydroxide. This trivalent (chelated or insoluble) chromium is a stable species and may be biologically unavailable to the nonfilter-feeding life stages that precede the prodissochonch I, straight-hinge stage (Mertz, 1969). Chromium (III) has been shown to impair the ciliary mechanism of the gill in adults of the mussel Mytilus edulis and the soft-shell clam Mya arenaria (Capuzzo, 1974; Chipman, 1966; Oshida et al., 1981). Although, in other investigations, Cr(III) was innocuous at concentrations as high as 50,400 ug/l (Oshida et al., 1981).

In view of these studies, the hard clam egg toxicity test should serve as a useful tool for identifying the potential impact of drilling muds on the survival of commercially important bivalve mollusc species in the offshore environment. As demonstrated in this study, very low concentrations of drilling muds can adversely affect the survival of newly fertilized eggs. In addition, this information should be useful in determining the maximum permissable mud discharge rates in coastal zones that serve as seasonal nurseries for commercially important off-shore species such as the sea scallop Placopecten magellanicus (Posgay and Norman, 1958; Naidu, 1970), the ocean quahog Arctica islandica (Loosanoff, 1953; Jones, 1981) and the surf clam Spisula solidissima (Ropes, 1968, Jones, 1981). Unlike offshore species such as the sea scallop P. magellanicus and the sea clam A. islandica which require a minimum of 4-8 days for the developing larvae to reach D-stage (Culliney, 1974 and Lutz et al., 1982), M. mercenaria fertilized eggs develop to this stage in 24-48 h, a distinct advantage in using this species for marine toxicity testing.

The nature and extent of the impact of oil drilling fluid discharges on molluscs and other marine organisms would depend on the time of year, the quantity and the frequency of the discharge. In addition, the hydrodynamics of the release site would be important in assessing the potential impact of drilling mud releases. Adverse effects associated with the long-term discharge of muds from several drilling operations in a small area could be greater than expected, since the sensitive, early life stages of many marine organisms can remain planktonic for at least several days (Culliney, 1974; Goldberg, 1980; Lutz _et_ _al_., 1982), thus prolonging their potential exposure.

In conclusion, while the hard clam fertilized egg stage has been shown to be sensitive to a variety of used drilling muds, it has not been possible to correlate toxicity conclusively with any specific mud components. Further toxicological studies are required to deliniate the effects of turbidity and particulate loading (barium and clay), the effects of adsorption of toxicant to particulates, and the role of microorganisms in the biodegration of the various drilling fluid components.

II. TRACE METALS IN DRILLING FLUID/SEA WATER TOXICITY TEST PHASES

2.1 Background

Trace metal analysis of the drilling fluid/sea water toxicity test phases was conducted in an attempt to identify inorganic components that may be toxic. The metals of interest include barium, cadmium, chromium, copper, manganese, nickel, lead and zinc. These metals can be present in the marine environment in several forms which will affect their availability and potential toxicity to marine organisms. For this reason a scheme was developed to "speciate" or determine the various forms of the trace elements listed above as well as their total concentrations in drilling fluid/sea water mixtures.

Three general types of metal species were targeted in the speciation scheme to be described. These were: free ionic forms including inorganic complexes (i.e., chloro, hydroxy, etc.), organically bound metals, and particulate associated metals. These classes of trace elements are considered to be the major types present and have been the subject of numerous speciation studies (Bender, 1982; Florence, 1982; Hart, 1982). Total concentrations give no information about a metal's availability and frequently show no significant correlation with toxicity (Allen *et al*., 1980). Metals adsorbed, occluded or in some way associated with colloidal-sized particulate matter may not be readily available to many types of marine organisms and are often assumed to be non-toxic (Florence, 1982). Metals complexed by organic ligands are known to be less toxic than free metal ions (Allen *et al*., 1980; Luoma, 1983; Zamada and Sunda, 1982).

In addition to the types of species discussed above, chromium can exist in two oxidation states in sea water (Cranston and Murray, 1978; Nakayama et al., 1981). Chromium(VI) is present as CrO_4^{2-} and is considered highly toxic while Cr(III) as $Cr(OH)_2^+ . 4H_2O$ is much less toxic (Carr et al., 1982; Mayer and Schick, 1981; Florence, 1982). Determination of the oxidation state and species of Cr was also included as part of the speciation scheme.

In order to clearly interpret results from the speciation of drilling mud/sea water mixtures, a knowledge of drilling mud components and additives is essential. The Ba in drilling muds is added in large amounts as barite ($BaSO_4$) which is insoluble. Barium is, therefore, expected to be mainly in a particulate form with a very small amount present as soluble Ba. Chrome or ferrochrome lignosulfonates are widely used as defloculants and are the source of Cr in drilling fluids (Perricone, 1980). It has also been reported that Cr(VI), as dichromate, is sometimes added to a drilling mud to regain lost thinning action or for corrosion control (Moseley, 1980). Lead may be present in drilling fluids as a contaminant from pipe dope used for drill strings (Liss et al., 1980; Kalil, 1980). Many of the other metals may be introduced into drilling fluids as impurities in clay, barite or other additives (Macdonald, 1982). Manganese for example, is often associated with clay minerals in significant quantities.

Drilling fluids also contain additives that can chelate trace metals. Lignosulfonates are polymeric lignin derivatives containing large numbers of sulfonic acid, carboxylic acid and phenolic groups that can bind metal ions. Although Cr(III) and Fe are probably principally associated with lignosulfonates, it is unlikely that the binding capacity of this ligand is completely expended in typical mud mixtures. Metals such as Cd, Cu, Mn, Ni, Pb and Zn are probably complexed to some extent when present in drilling muds. Lignite is another additive that can have metal complexing components. Lignite contains humic acid and related compounds (Perricone, 1980) that are well known naturally occurring metal chelators (Mantoura *et al.*, 1978).

2.2 Materials and Methods

2.2.1 Particulate and Dissolved Metals.

To obtain information on particulate, dissolved, and free metal ion concentrations, individual aliquots of both the liquid (72 h settled) and suspended solids (1 h settled) test phases where taken and treated separately. For each drilling mud a range of concentrations was prepared for toxicity testing, but only the most concentrated sample of each type of test phase (typically 3 mL drilling mud/L sea water) was sampled for trace metal analysis. Four 5 mL portions were pipetted from each test phase. Two of the 5 mL aliquots were centrifuged for 10 min at approximately 3000 x G. Analysis of the uncentrifuged sample gave total metal concentrations while the centrifuged samples were used to measure dissolved metal concentrations. The difference between the two was considered particulate metal concentration.

2.2.2 Free Metal.

Free metal ion concentrations in the drilling fluid/sea water mixtures were determined by performing equilibrium dialysis (ED) separations prior to trace metal analysis. Dialysis experiments were performed in duplicate on 200 or 250 mL aliquots by using two types of membranes. Nominal 1,000 molecular weight cut-off (MWCO) tubing (Spectrum Medical Industries, Spectra/Por 6) was cleaned by the method of Truitt and Weber (1981a) and sealed at the ends with plastic closures to form dialysis bags. Ion exchange membranes (RAI Research Corp, Millipore R-1010) were prepared according to Blaedel and Kissel (1972) and used with dialysis cells made from polyethylene bottles approximately 1.8 cm in diameter. After removing the bottoms, the bottles were fitted with membranes held in place with rubber "O" rings. The dialysis cells or bags were filled with 15 mL of 0.45 um filtered sea water used as the toxicity test control.

Dialysis was performed in 250 mL polyethylene centrifuge bottles containing the drilling fluid/sea water test phases. The bottles were placed on a rotary shaker at low speed (75 rev/min). After a predetermined amount of time, the dialysis cells or bags were removed and sampled for trace metal analysis. Additional samples were checked for UV absorbance at 275 nm to detect the presence of lignosulfonate or other UV-absorbing organic compounds. In addition to analyzing the internal dialysis solution, the test phase external to the cell was analyzed. Prior to sampling, the external solution was centrifuged for 20 minutes at approximately 2,500 x G. The internal solution gave free metal concentration while the external solution, after centrifugation, gave total dissolved metal concentrations. The difference between the two was considered bound or complexed metal concentration.

Initially ED experiments were conducted on aqueous (free) metal ion solutions alone. These experiments were designed to determine the amount of time needed to attain equilibrium across the membranes in the sea water medium.

2.2.3 Chromium Speciation.

Additional experiments were needed to determine what fraction, if any, of the free Cr measured in ED experiments was present as Cr(III). To achieve this, Donnan dialysis (DD) was employed. The ion exchange membranes described for ED were also used in these experiments but the dialysis cells were loaded with 15.0 mL of 1 M HNO_3 to promote DD. Initial DD experiments were done in triplicate with Cr(III) or Cr(IV) only, at three concentrations. Sample aliquots were taken 2, 3, 4 or 6 h after the start of dialysis. The desired result was to maximize transport of Cr(III) across the membranes while minimizing Cr(VI) transport. Once optimum conditions were determined, experiments were conducted with selected drilling fluid/sea water phases.

2.2.4 Trace Metal Analysis.

All solutions for trace metal analysis were stored and transferred by using acid-cleaned plasticware. The samples for particulate and dissolved metal analysis were analyzed within two hours of sampling. The uncentrifuged samples were shaken periodically prior to analysis to keep particles suspended. All other sample aliquots (i.e., from dialysis) were preserved with 5 uL/mL of redistilled HNO_3.

Eight elements (Ba, Cd, Cr, Cu, Mn, Ni, Pb and Zn) were determined simultaneously by direct current plasma emission spectrometry (DCP) with a Spectrametrics Spectraspan IIIB. Sample emission intensities were compared with a two-point calibration curve of standards in acidified and 0.45 um filtered sea water. Data aquisition and calculations, including instrument drift correction, were performed by a dedicated minicomputer (Charles River Data Systems, model MF-211).

2.3 Results and Discussion

2.3.1 Preliminary Studies.

It was necessary to test certain aspects of the speciation procedure on laboratory-prepared solutions before application to drilling fluids. The 1,000 MWCO dialysis membranes nave been used in speciation studies in freshwater and were demonstrated to adequately separate trace metals and certain natural ligands via equilibrium dialysis (ED) (Truitt and Weber, 1981a, 1981b; Rainville and Weber, 1982). These membranes have not been used in a sea water medium or for all the metals studied here. Initial experiments demonstrated that equilibrium was reached between 18 and 24 hours after the start of dialysis for all eight metals tested. This included both Cr(III) and Cr(VI).

The ion exchange membranes were used in two modes, for ED and Donnan dialysis (DD). For ED the sample was dialyzed against a similar medium (0.45 um filtered sea water) and equilibrium was reached slowly. The ion exchange membranes contain sulfonic acid groups ($-SO_3H$) that are dissociated and negatively charged at the pH of sea water. The porous nature of the membranes allow small ions to pass under ED conditions (i.e., same medium on both sides), but large anionic molecules such as lignosulfonate or lignite are repelled by the sulfonate groups. It was determined that approximately 100 hours were necessary for equilibrium to be reached with metal ions alone. Chromium(VI), however, did not reach equilibrium over this time period because of its anionic form. For this reason, the ion exchange membranes could not be used for ED of Cr(VI). Dialysis of ferrochrome lignosulfonate (Q-Broxin, Baroid Corp.) demonstrated that 90 to 100% of the lignosulfonate was rejected by the membranes. Lignosulfonate was quantified by measuring UV absorbance of the peak at 275-280 nm (Alberts, 1982, Almgren *et al*., 1975). This was done for the dialysis of test phases as well. Only 0 to 7% of the UV-absorbing species were able to pass the membranes.

Donnan dialysis is a relatively rapid process compared to ED and it is possible to achieve an enrichment of the analyte in the dialysis cell (Cox and Twardowski, 1980). In DD the sample is dialyzed against a high concentration of a particular cation. Experiments with Cr(III) in sea water dialyzed against 1 M HNO_3 exhibited increasing DD of Cr(III) after 2, 3, and 4 h. At 6 h, however, the DD process had ceased and ED was occurring. Under the same conditions Cr(VI), because of its anionic form, does not undergo DD. It instead gradually permeates the membrane as in ED, causing a slow increase in Cr(VI) concentration in the cell. Since the desired result was to use DD to determine Cr(III), a 2 h dialysis time was chosen. This allowed a slight enrichment of Cr(III) in the cell but only a small contamination of Cr(VI). Triplicate experiments with Cr(III) gave an enrichment factor of 1.38 and a relative standard deviation of 2.2%. Under the same conditions, equivalent amounts of Cr(VI) caused an 8% contamination. This means that DD of a mixture of 0.100 ppm Cr(III) and 0.100 ppm Cr(VI) would yield 0.138 ppm of Cr(III) and 0.008 ppm of Cr(VI) in the dialysis cell. It was concluded that these results were adequate for the technique to be useful for speciation of Cr in drilling muds.

2.3.2 Test Phase Results.

The results of trace metal analysis and speciation of liquid and suspended-solids test phases are listed in Tables III through VII for Ba, Cr, Cu, Mn and Zn. When comparing results for different drilling fluids, the sea water dilution factors (test phase concentration) must be considered. The concentrations of metal are listed under three categories. Total metal includes all forms: particulate, free and organically bound. Solution phase metals are dissolved forms, both free and bound, from analyses of centrifuged samples. Free metal is the concentration that can pass the ED membranes and is assumed to be small inorganic species.

The concentrations of Cd, Ni and Pb in the muds diluted with sea water were undetectable. Detection limits for the DCP system were 0.01, 0.02 and 0.20 ppm for Ni, Cd and Pb respectively. The results for the Sept. 4 Mobile Bay mud that was used as a quality control toxicity standard are shown at the end of each table (III-VII). These data give a realistic idea of the day-to-day precision of preparation and sampling of the test phases.

Table III. CONCENTRATION AND SPECIATION OF BARIUM IN DRILLING FLUID/SEAWATER TEST PHASES

Drilling Fluid	Type of Phase[a]	Phase Conc.[b] (mL/L)	Concentration of Barium (mg/L) Total	Solution	Free
AN31	Liquid	3.0	0.25 ±0.01	0.104±0.002	0.098±0.002
	Suspended	2.5	3.1 ±0.5	0.120±0.002	
MIBLKA51	Liquid	3.0	0.093±0.004	0.082±0.002	0.078±0.002
	Suspended	5.0	1.8 ±0.3	0.071±0.002	
SV76	Liquid	3.0	0.7 ±0.4	0.13 ±0.01	0.117±0.003
	Suspended	0.15	2.61 ±0.07	0.130±0.01	
P1	Avg.Liq.(2)	3.0	0.4 ±0.2	0.087±0.002	0.09 ±0.02
	Suspended	1.0	5.5 ±0.1	0.034±0.003	0.067±0.009[c]
P2	Avg.Liq.(2)	3.0	0.34 ±0.05	0.124±0.004	0.112±0.005
	Suspended	0.5	5.3 ±0.1	0.07 ±0.001	0.049±0.002
P3	Liquid	2.0	0.85 ±0.06	0.153±0.008	0.092±0.002[c]
	Liquid	3.0	1.23 ±0.09	0.28 ±0.01	
	Suspended	1.0	9.0 ±0.9	0.27 ±0.07	0.2 ±0.2[c]
P4	Liquid	3.0	0.43 ±0.02	0.29 ±0.02	0.092±0.002
	Suspended	0.5	13.5 ±0.9	0.87 ±0.01	0.2 ±0.02[c]
P5	Liquid	3.0	1.16 ±0.03	0.158±0.003	0.098±0.006
	Suspended	0.5	6.4 ±0.8	0.119±0.005	0.09 ±0.01
P6	Liquid	3.0	0.58 ±0.003	0.052±0.002	0.067±0.003[c]
	Avg.Sus.(2)	3.0	0.4 ±0.3	0.050±0.003	0.06 ±0.01[c]
P7	Liquid	3.0	0.152±0.009	0.109±0.001	0.131±0.005[c]
	Avg.Sus.(2)	3.0	5 ±3	0.12 ±0.01	0.14 ±0.04[c]
P8	Avg.Liq.(2)	3.0	0.7 ±0.1	0.183±0.006	0.15 ±0.02[c]
	Avg.Sus.(2)	3.0	23 ±14	0.4 ±0.3	0.20 ±0.01
Sept. 4 (Mobile Bay)	Avg.Liq.(3)	1.0	0.4 ±0.2	0.033±0.003	0.04 ±0.02[c]
	Avg.Sus.(4)	0.5	8 ±3	0.028±0.011	0.10 ±0.08[c]

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[a] Replicate phases are expressed as an average with number of replicates in parentheses.

[b] Concentrations are mL of whole mud per L of 0.45 um filtered seawater.

[c] There was no significant difference between free and solution phase barium in these experiments.

Table IV. CONCENTRATION AND SPECIATION OF CHROMIUM IN DRILLING FLUID/SEAWATER TEST PHASES

Drilling Fluid	Type of Phase[a]	Phase Conc.[b] (mL/L)	Concentration of Chromium (mg/L)		
			Total	Solution	Free
AN31	Liquid	3.0	0.038±0.002	0.034±0.002	0.017±0.002[c]
	Suspended	2.5	0.105±0.009	0.036±0.004	
MIBLKA51	Liquid	3.0	0.010±0.002	0.010±0.002	0.005±0.002[c]
	Suspended	5.0	0.076±0.005	0.016±0.003	
SV76	Liquid	3.0	0.59 ±0.02	0.543±0.004	0.214±0.007[c]
	Suspended	0.15	0.135±0.008	0.051±0.003	
P1	Avg.Liq.(2)	3.0	0.155±0.004	0.15 ±0.02	0.067±0.002[c]
	Suspended	1.0	0.110±0.003	0.037±0.003	
P2	Avg.Liq.(2)	3.0	0.044±0.005	0.041±0.003	0.025±0.002[c]
	Suspended	0.5	0.083±0.003	0.016±0.003	
P3	Liquid	2.0	0.110±0.006	0.093±0.003	d
	Liquid	3.0	0.12 ±0.02	0.11 ±0.01	
	Suspended	1.0	0.115±0.009	0.037±0.004	
P4	Liquid	3.0	0.339±0.008	0.341±0.005	d
	Suspended	0.5	0.134±0.005	0.056±0.004	
P5	Liquid	3.0	0.039±0.003	0.033±0.003	d
	Suspended	0.5	0.014±0.003	0.008	
P6	Liquid	3.0	0.004	0.004	d
	Avg.Sus.(2)	3.0	0.004	0.004	
P7	Liquid	3.0	0.007±0.002	0.006±0.002	d
	Avg.Sus.(2)	3.0	0.09 ±0.03	0.009±0.002	
P8	Avg.Liq.(2)	3.0	0.27 ± 0.02	0.264±0.008	0.031±0.006[c]
	Avg.Sus.(2)	3.0	0.6 ± 0.2	0.275±0.003	
Sept. 4 (Mobile Bay)	Avg.Liq.(3)	1.0	0.9 ± 0.2	0.8 ±0.2	0.076±0.002
	Avg.Sus.(4)	0.5	1.3 ±0.5	0.6 ±0.2	0.11 ±0.03

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a Replicate phases are expressed as an average with number of replicate in parentheses.

b Concentrations are mL of whole mud per L of 0.45 um filtered seawater.

c Used 1000 MWCO membranes for one test phase in duplicate; see text for discussion.

d Not determined.

Table V. CONCENTRATION AND SPECIATION OF COPPER IN DRILLING FLUID/SEAWATER TEST PHASES

Drilling Fluid	Type of Phase[a]	Phase Conc.[b] (mL/L)	Concentration of Copper (mg/L)		
			Total	Solution	Free
AN31	Liquid	3.0	0.004±0.002	0.004±0.002	0.003±0.002
	Suspended	2.5	0.007	0.007	
MIBLKA51	Liquid	3.0	0.003±0.001	0.002±0.001	0.002±0.002
	Suspended	5.0	0.013±0.004	0.002	
SV76	Liquid	3.0	0.043±0.002	0.041±0.002	0.010±0.002
	Suspended	0.15	0.007	0.007	
P1	Avg.Liq.(2)	3.0	0.009±0.002	0.007±0.003	0.005±0.002
	Suspended	1.0	0.004±0.001	0.003±0.001	0.006
P2	Avg.Liq.(2)	3.0	0.002±0.001	0.002±0.001	0.003±0.002
	Suspended	0.5	0.006	0.004	0.006
P3	Liquid	2.0	0.011±0.003	0.009±0.003	0.004
	Liquid	3.0	0.015±0.002	0.014±0.002	
	Suspended	1.0	0.010±0.003	0.005±0.003	0.006
P4	Liquid	3.0	0.004	0.004	0.004
	Suspended	0.5	0.006	0.006	0.006
P5	Liquid	3.0	0.012±0.003	0.013±0.003	0.004
	Suspended	0.5	0.005	0.005	0.005
P6	Liquid	3.0	0.004	0.004	0.004
	Avg.Sus.(2)	3.0	0.004	0.004	0.004
P7	Liquid	3.0	0.004	0.004	0.004
	Avg.Sus.(2)	3.0	0.004±0.002	0.004	
P8	Avg.Liq.(2)	3.0	0.006±0.002	0.006±0.002	0.003±0.002
	Avg.Sus.(2)	3.0	0.016±0.008	0.004±0.004	0.004
Sept. 4 (Mobile Bay)	Avg.Liq.(3)	1.0	0.013±0.002	0.011±0.003[c]	0.004
	Avg.Sus.(4)	0.5	0.013±0.003	0.005±0.002[c]	0.005

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[a] Replicate phases are expressed as an average with number of replicates in parentheses.

[b] Concentrations are mL of whole mud per L of 0.45 um filtered seawater.

[c] Average was computed using only the values above the detection limit.

Table VI. CONCENTRATION AND SPECIATION OF MANGANESE IN DRILLING FLUID/SEAWATER TEST PHASES

Drilling Fluid	Type of Phase[a]	Phase Conc.[b] (mL/L)	Concentration of Manganese (mg/L)		
			Total	Solution	Free
AN31	Liquid	3.0	0.03 ±0.01	0.027±0.007	0.036±0.007
	Suspended	2.5	0.029±0.005	0.014±0.005	
MIBLKA51	Liquid	3.0	0.01 ±0.01	0.01 ±0.01	0.02
	Suspended	5.0	0.014±0.006	0.012	
SV76	Liquid	3.0	0.27 ±0.01	0.27 ±0.01	0.23 ±0.01
	Suspended	0.15	0.06 ±0.01	0.021±0.009	
P1	Avg.Liq.(2)	3.0	0.04 ±0.01	0.04 ±0.01	0.03 ±0.01
	Suspended	1.0	0.018±0.005	0.010±0.004	0.02
P2	Avg.Liq.(2)	3.0	0.02	0.02	0.02
	Suspended	0.5	0.012±0.005	0.01	0.02
P3	Liquid	2.0	0.12 ±0.02	0.114±0.009	0.104±0.007
	Liquid	3.0	0.15 ±0.02	0.15 ±0.02	
	Suspended	1.0	0.060±0.007	0.023±0.004	0.08 ±0.01[c]
P4	Liquid	3.0	0.23 ±0.01	0.22 ±0.01	0.19 ±0.01
	Suspended	0.5	0.028±0.005	0.017±0.005	0.05 ±0.01[c]
P5	Liquid	3.0	0.10 ±0.01	0.093±0.008	0.084±0.008
	Suspended	0.5	0.026±0.006	0.007±0.005	0.02
P6	Liquid	3.0	0.041±0.003	0.037±0.007	0.04 ±0.01
	Avg.Sus.(2)	3.0	0.026±0.008	0.021±0.000	0.03 ±0.01[d]
P7	Liquid	3.0	0.008	0.008	
	Avg.Sus.(2)	3.0	0.03 ±0.01	0.011±0.003	0.002[d]
P8	Avg.Liq.(2)	3.0	0.11 ±0.02	0.10 ±0.01	0.106±0.008[d]
	Avg.Sus.(2)	3.0	0.10 ±0.04	0.055±0.008	0.047±0.006[d]
Sept. 4 (Mobile Bay)	Avg.Liq.(3)	1.0	0.03 ±0.01	0.021±0.008	0.015±0.007[d]
	Avg.Sus.(4)	0.5	0.07 ±0.02	0.020±0.008	0.024±0.009

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[a] Replicate phases are expressed as an average with number of replicates in parentheses

[b] Concentrations are mL of whole mud per L of 0.45 um filtered seawater.

[c] Slight contamination but no difference between free or solution phase manganese.

[d] One test phase analyzed in duplicate

Table VII. CONCENTRATION AND SPECIATION OF ZINC IN DRILLING FLUID/SEAWATER TEST PHASES

Drilling Fluid	Type of Phase[a]	Phase Conc.[b] (mL/L)	Concentration of Zinc (mg/L)		
			Total	Solution	Free
AN31	Liquid	3.0	0.008	0.008	0.006
	Suspended	2.5	0.015±0.004	0.008	
MIBLKA51	Liquid	3.0	0.004±0.003	0.006	0.008
	Suspended	5.0	0.019±0.005	0.010	
SV76	Liquid	3.0	0.022±0.003	0.010±0.003	0.017±0.003
	Suspended	0.15	0.027±0.006	0.010	
P1	Avg.Liq.(2)	3.0	0.005±0.003	0.005±0.003	0.005±0.003
	Suspended	1.0	0.019±0.002	0.007±0.002	0.017±0.007[c]
P2	Avg.Liq.(2)	3.0	0.008	0.008	0.008
	Suspended	0.5	0.034±0.008	0.004	0.013±0.007[c]
P3	Liquid	2.0	0.009±0.004	0.008±0.003	0.014±0.003[c]
	Liquid	3.0	0.008	0.008	
	Suspended	1.0	0.027±0.004	0.007±0.002	0.011±0.006
P4	Liquid	3.0	0.017±0.004	0.013±0.004	0.039±0.004[c]
	Suspended	0.5	0.042±0.004	0.007±0.004	0.019±0.004[c]
P5	Liquid	3.0	0.008	0.008	0.019±0.006[c]
	Suspended	0.5	0.006	0.008	0.013±0.004[c]
P6	Liquid	3.0	0.018±0.003	0.017±0.006	0.043±0.008[c]
	Avg.Sus.(2)		0.013±0.004	0.013±0.005	
P7	Liquid	3.0	0.006	0.006	
	Avg.Sus.(2)		0.012±0.002	0.006	0.22 ±0.05[d]
P8	Avg.Liq.(2)	3.0	0.007±0.003	0.008±0.005	0.005±0.003
	Avg.Sus.(2)	3.0	0.06 ±0.03	0.011±0.003	0.21 ±0.01[c,d]
Sept. 4 (Mobile Bay)	Avg.Liq.(3)	1.0	0.072±0.008	0.040±0.005	0.02 ±0.01
	Avg.Sus.(4)	0.5	0.23 ±0.09	0.05 ±0.02	0.04 ±0.02

---

[a] Replicate phases are expressed as an average with number of replicates parentheses.

[b] Concentrations are mL of whole mud per L of 0.45 um filtered seawater.

[c] Slight contamination but no difference between free and solution phase zinc in these experiments.

[d] One test phase analyzed in duplicate.

2.3.3 Barium.

The concentrations of Ba in the test phases (Table III) were the highest of any element determined. Liquid phase results show that even after 72 hours of settling time, Ba suspended in the water column was still significantly higher than dissolved Ba. Total concentrations were higher than solution concentrations in every case. This was probably due to colloidal $BaSO_4$.

The high and variable total concentrations were expected for suspended-solids phase Ba because a great deal of particulate $BaSO_4$ was still suspended after 1 hour of settling. Rough calculations based on the Stoke's Law settling velocities of quartz spheres indicate that only particles less than 10 um in diameter would remain suspended in these phases. This is an upper limit for $BaSO_4$ because its density is almost twice that of quartz. The poor instrumental precision obtained for Ba in suspended-solids phases may be due to $BaSO_4$ crystallizing to a limited extent in the instrument nebulizer. The accuracy of the measurement for these particles is probably still good. It has been demonstrated that particles up to 14 um are atomized with virtually 100% efficiency by using the DCP system (Saba et al., 1981).

Solution phase Ba numbers were similar for liquid and suspended-solids phases of a given mud even though for some muds the two phases were of different concentration. These values varied somewhat from mud to mud and were significantly higher than the published sea water solubility limit for $BaSO_4$ (Chow and Goldberg, 1960) with the possible exceptions of the P6 and Sept. 4 muds. Barium can exist in solution above its sea water solubility because certain mud components in these complex mixtures can alter the solubility equilibria involved.

Data for free Ba concentrations were very similar to solution phase values, but were slightly lower in some cases. This indicates that the predominant form of this element in solution was Ba^{2+}, but a small amount could have been soluble as ion pairs of $BaSO_4$.

2.3.4 Chromium.

Particulate Cr was a significant portion of the total concentrations only for the suspended-solids phase, with total values ranging from three to five times the solution concentrations (Table IV). The only exception was the P7 suspended solids phase which had a particulate Cr concentration that was ninefold higher than the solution concentration of Cr. Total Cr values for the liquid phases were essentially the same as solution Cr values. Solution concentrations were similar for both types of test phases when phase concentration is considered.

As mentioned above, the ion exchange membranes were unsatisfactory for ED of Cr(VI). The 1000 MWCO membrane results were the only data used to obtain free Cr values. For this reason, the data in the "free" column of Table IV are limited. A significant difficulty with these membranes, however, is that they are not as good as the ion exchange membranes for preventing the passage of the organic ligands. In experiments with the 1000 MWCO membranes, an average of 40% of the UV absorbing material from the test phases passed through the membranes with the free ions. This means that some bound Cr may pass the membranes so the results could overestimate the free ion concentrations. Although this possibility exists, it is not likely that this overestimate is very large. Results for Ba, Cu, Mn and Zn showed agreement between membranes. This is possible even in light of the UV data because these measurements are not specific for the organic ligands and may measure other UV absorbing components of the phases that do not bind metal ions.

2.3.5 Free Chromium(III) vs Chromium(VI).

Donnan dialysis (DD) of selected test phases separated free Cr(III) from free Cr(VI) and bound Cr. The results are shown in Table VIII. Free Cr(VI) was calculated by difference from the total free Cr data (Table IV) and the free Cr(III) values. In most cases the Cr(VI) values were very low or undetectable; however, for the SV76 and P1 test phases, the Cr(VI) concentration was significant. To confirm the dialysis results, Cr(VI) in the SV76 and P1 test phases was also determined by differential pulse polarography (DPP). The value determined for SV76 was 0.1 ppm, but Cr(VI) in the P1 test phase (3.0 mL/L) was below the detection limit of approximately 0.02 ppm for the conditions used. Both of the values determined by DPP were lower than the corresponding DD results. The reason for this is the overestimate of free Cr discussed above (Section 2.3.4). This overestimation may be by a factor of two, judging from this limited data. DPP results are not without potential interferences that could affect accuracy especially in a high organic matter matrix like drilling muds (Jacobson and Lindseth, 1976). However, results from these two independent methods indicate that Cr(VI) was definitely present in the SV76 mud and possibly in P1 as well. Information concerning the composition and history of the drilling muds used in this study indicate that Cr(VI) as dichromate was added to the SV76 mud at a substantial level (0.2 lbs/bbl) during drilling operations. Since Cr(VI) has been demonstrated to be the most toxic form of Cr to marine organisms (Carr *et al*., 1982; Mayer and Schick, 1981), and to certain larvae (Bookhout *et al*., 1982), it is likely that the concentrations of Cr(VI) in these two phases contribute to their toxicity. Of the phases that were tested, the SV76 liquid phase was found to be the most toxic to *M. mercenaria* larvae (Table I, Section 1), and the P1 mud was the most toxic to mysid shrimp (personal communication, Thomas W. Duke, EPA Gulf Breeze).

2.3.6 Copper.

The total Cu concentrations in all test phases were extremely low and were below detection limits in many cases (Table V). The SV76 test phases had the highest amount of Cu, with approximately 0.04 mg/L. Solution concentrations of Cu were similar to total values in general, with a few exceptions for suspended solids phases (MIBLKA51, P8, and Sept. 4 muds). Free Cu concentrations were less than 0.006 mg/L for all but the SV76 liquid phase. These data indicate that Cu occurs principally in a bound form in these muds. This is what would be expected, since Cu is known to form strong associations with organic ligands (Ryan and Weber, 1982; Mantoura *et al*., 1978).

TABLE VIII. FREE CHROMIUM (III) AND CHROMIUM (VI) FROM DONNAN DIALYSIS OF DRILLING FLUID/SEAWATER LIQUID PHASES[a]

Drilling Fluid	Concentrations of Chromium (mg/L)		
	Free Cr[b]	Free Cr(III)	Cr(VI) by difference[c]
AN31	0.017±0.002	0.022±0.006	0
MIBLKA51	0.005±0.002	0.026±0.006	0
SV76	0.214±0.007	0.025±0.007	0.19 ±0.01
P1	0.067±0.002	0.020±0.004	0.047±0.004
P2	0.025±0.002	0.014±0.007	0.011±0.007
P8	0.031±0.006	0.022±0.009	0.009±0.009

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[a] All phases were 3.0 mL of drilling fluid per L of 0.45 um filtered seawater.

[b] From Table IV.

[c] Cr(VI) concentrations are the difference between free Cr and Cr(III) values.

2.3.7 Manganese.

The results for Mn were quite consistent among total, solution and free values for the liquid phase (Table VI). The suspended-solids phase exhibited a degree of particulate Mn with solution and free values similar in most cases. These results show that in addition to the low particulate quantities, most of the Mn is present in the free form. Manganese, however, is not known to be highly toxic. Even though the concentrations of Mn reported here are substantially above the concentrations measured for coastal waters, there is probably little danger to marine organisms from Mn in drilling fluid discharges.

2.3.8 Zinc.

A small fraction of the total Zn measured for suspended-solids phases was present as a particulate form. The remainder of the Zn was present in a free form in these phases and the liquid phases (Table VII).

Difficulties were encountered with contamination of Zn in the dialysis experiments. Zinc is prevalent in urban environments and the source of the problem is believed to be the 0.45 um filtered sea water which is pumped from Boston Harbor. The contamination, however, does not preclude the conclusion that Zn is primarily free in the drilling muds that were analyzed. The justification for this is that the contaminant Zn was found to be between zero and three times the original concentration. Typically, any Zn present will partition to some extent, and may associate with particles or organic ligands. The dialysis experiment allows measurement of the final equilibrium state attained. Since all of the solution phase Zn was free, this is assumed to be the predominant form of the original Zn.

2.3.9 Conclusions

Results from the measurement of trace metals in drilling fluid-sea water mixtures showed that the average concentrations of the detectable elements decreased in the order Ba > Cr > Mn > Zn > Cu. The concentrations of Cd, Ni and Pb were below the detection limits of the measurement system (0.02, 0.01 and 0.2 mg/L respectively). All metals exhibited some particle association in 1 h settled phases (suspended solids) with Ba being present principally in the particulate form. Chromium and Cu were bound, probably as lignosulfonate complexes, but Mn and Zn were primarily in free forms. A significant portion of the Cr was present as highly toxic Cr(VI) in two of six muds analyzed for this form of Cr.

The potential threat of metal toxicity, bioaccumulation and food chain biomagnification with respect to marine organisms is greater from Cr than other elements tested. Although most of the Cr is probably present as Cr(III) complexes of lignosulfonate, its concentration is relatively high. The lack of toxicity usually observed for Cr(III) has been attributed to its low solubility (Carr et al., 1982). Lignosulfonate complexes Cr(III) and increases its solubility, thus increasing the potential threat of this form of Cr (Liss et al., 1980; Knox, 1978). In addition Cr(VI) is often the form of Cr added to lignosulfonate to prepare chrome or ferrochrome lignosulfonate (Knox, 1978). Chromium(VI) salts are sometimes added to drilling muds as well (Moseley, 1980; Section 2.3.5). Considering the large quantities that are used, it is very likely that some Cr(VI) will remain unreacted in the mud where pH conditions are unfavorable for Cr(VI) reduction (Moseley, 1980). In oxygenated sea water, the stable form of dissolved Cr is Cr(VI). It has been demonstrated that Cr(III) is present, but slow oxidation occurs in the presence of O_2 and is catalyzed by manganese oxide (Van der Weijden and Reith, 1982). This suggests that any Cr inputs to the ocean, regardless of the form, are potentially harmful.

III. ORGANIC CONSTITUENTS IN WHOLE DRILLING FLUID AND DRILLING FLUID/SEA WATER TOXICITY TEST PHASES

3.1 Background

Since there is a paucity of general information in the literature on drilling fluids and their specific chemical constituents (Sprague and Logan, 1979), it was considered necessary to perform qualitative/quantitative analysis of organic constituents. The possibility that the organic constituents of the used drilling fluids may contribute to the various toxicological responses of marine organisms is of important consideration. Development of methodologies for organic analyses of the used whole drilling muds and the drilling mud test phase solutions was conducted by modifying accepted organic analysis techniques (IERL-RTP Procedure Manual: Level 1; Environmental Assessment, U.S. EPA, 1978). These procedures were employed to provide a satisfactory characterization of the whole mud and test phases by identifying the major classes of organic compounds and their concentrations.

Qualitative/quantitative analyses were directed specifically toward the analysis of petroleum hydrocarbons in drilling fluids. The presence of hydrocarbons indicates either their intentional addition to drilling fluids to aid in the drilling process or their natural occurrence in strata penetrated by the drill (Grahl-Nelson *et al*., 1980; Neff, 1981). There is an abundance of data on physiological responses of marine organisms to petroleum hydrocarbons; knowledge of the typical addition of diesel oils (#2 fuel oil) to drilling fluids warranted the analysis and quantification of petroleum hydrocarbons in drilling fluids.

3.2 Materials and Methods

3.2.1 Whole Drilling Fluid Analyses.

Quantitative and qualitative determinations of organic compounds (i.e., #2 fuel oil components) in whole drilling muds were made by following standard modified methodologies (previously referenced, Sec. 3.1). All glassware was precleaned by either solvent rinsing or heating at 500°C. Solvents used in extraction and chromatography procedures were of "distilled-in-glass" purity, and the chemical adsorbents (magnesium sulfate and silica gel) were pre-cleaned by either solvent extraction or heating at 500°C.

Initially, a 25.0 g aliquot of drilling mud was weighed in a beaker, and the pH of the sample was adjusted to 2.0 ± 0.5 with hydrochloric acid. After 0.5 h equilibration in an ice bath, magnesium sulfate was added with mixing to adsorb the water and provide a homogeneous mixture for extraction. The mixture was then extracted in a Soxhlet-extraction apparatus for 24 h by using 250 mL of methylene chloride. Next, the methylene chloride extract was concentrated to 5.0 mL in a Kuderna-Danish concentrator on a steam bath, and a 2.0 mL portion of the concentrated extract was pipetted into a glass chromatographic column (300 mm x 10.5 mm) packed with a 6.0 g portion of freshly activated silica gel in 50% v/v methylene chloride/pentane. The sample was eluted with 50 mL methylene chloride/pentane (50% v/v) at a flow rate of 1-2 mL/min to elute petroleum hydrocarbon aliphatic and aromatic fractions. The eluent was concentrated to 5 mL by using steam or rotary evaporation and then adjusted to 5.0 mL for gas chromatographic/mass spectrometric analysis.

Gas chromatographic/mass spectometric (GC/MS) analyses were performed by using a Hewlett-Packard 5992A GC/MS with data system. Chromatographic separations were carried out by using 180 cm x 2 mm i.d. glass columns packed with 10% SP-2100 on Supelcoport 100/120 mesh, and with temperature programming. The mass spectrometer was operated in the electron-impact mode, and low resolution mass spectra were obtained by continuous scanning under control of the data system. Quantification was based on the measurements of external standards (i.e., API #2 fuel oil).

3.2.2 Drilling Fluid/Sea water Test Phases.

One-liter aliquots of the drilling fluid-sea water test phases were sampled, and 100 mL of methylene chloride was added to each sample for preservation of sample integrity until extraction. The liquid sample was transferred to a 2 L separatory funnel and was shaken vigorously for approximately 2 min. The methylene chloride extract was drawn off. A second 100 mL volume of methylene chloride was added to the sample for an additional 2 min. extraction. The combined methylene chloride extracts were dried over anhydrous sodium sulfate and concentrated to 10 mL by rotary evaporation under reduced pressure or by steam. After transfer to calibrated sample tubes, the sample volume was further reduced to 100-500 uL by air blow-down. Various aliquots of the sample concentrate were analyzed by previously described GC/MS procedures.

Bulk characteristics of the whole drilling muds were determined by a variety of procedures. Percentage water (% H_2O) was determined by overnight drying of a whole mud subsample at 105°C.

The percentage weight loss was determined gravimetrically. The pH of each sample was measured by mixing a 1:5 ratio, whole mud sample:distilled water, and determining the pH potentiometrically. Density (g/mL) was determined by the weight determination of a specific volume of whole mud. Organic volatiles (mg/g) were determined by combusting dried whole mud samples at 550°C for 1 h. The weight loss was determined gravimetrically.

3.3 Results and Discussion

For organic constituent analyses, the whole drilling muds containing 25-70% water (Table IX) could not be treated as aqueous solutions. Extraction techniques (i.e. mixing the drilling fluid with a polar extraction solvent like methylene chloride) produced an emulsion which could not be separated satisfactorily. An alternative approach was to freeze dry the drilling fluid sample and to extract the residue with solvents to isolate the organic constituents. However, freeze drying caused the loss of volatile organic components through co-distillation processes and proved this method to be unsatisfactory. Finally, a more satisfactory method was used that involved the addition of an excess of anhydrous magnesium sulfate ($MgSO_4$) to dehydrate the drilling fluid, giving a powdery mixture from which organic constituents were readily extracted with methylene chloride.

Silica gel column chromatography proved suitable for sample clean-up and separation. Gas chromatography/mass spectrometry analysis (GC/MS) was performed on these fractions to identify the principal organic-extractable constituents of the whole drilling

TABLE IX. BULK CHARACTERISTICS OF WHOLE DRILLING FLUID

Drilling Fluid	pH	%H2O	Density g/mL	Density lb/gal	Volatiles (dry weight, mg/g
AN31	10.5	49.9	1.50	12.5	50.8
MIBLKA51	10.0	69.5	1.26	10.5	38.5
SV76	10.5	27.3	2.17	18.1	31.4
P1	11.9	33.8	1.93	16.1	37.6
P2	12.1	30.0	2.02	16.9	37.6
P3	11.2	26.8	2.19	18.3	34.5
P4	8.0	33.5	1.93	16.1	20.5
P5	11.5	26.3	2.20	18.4	16.7
P6	8.8	71.5	1.22	10.2	26.6
P7	11.4	57.0	1.37	11.4	44.2
P8	10.4	27.3	2.17	18.1	21.0
Sept.4 (Mobile Bay)	9.7	54.2	1.55	12.9	49.9

fluid. GC/MS scans of the whole drilling fluid extracts closely resembled those of fuel or diesel oils. These results are presented in Table X as #2 fuel oil (mg/g). Presence of an unresolved complex mixture (UCM) and an n-alkane homologous series of C_{12} - C_{22} together indicate the hydrocarbons were of petrogenic origin. It should be noted that some of the lower molecular weight (earlier eluting) components were of lower concentration than found in #2 fuel oil. Also, the unresolved mixture shifted to longer retention times.

Additional information detailing bulk characteristics of the whole drilling fluids are presented in Table IX. Percentage water, pH, density, and organic volatiles were determined for the whole muds. These data detail various physical characteristics of the drilling fluids.

The drilling mud-sea water test phases (liquid and suspended solids) were more easily analyzed than the whole drilling fluids for organic constituents. Toxicity test solutions were extracted with polar solvents to isolate organic-extractable components. After concentration of the extracts, GC/MS analyses presented qualititative/quantitative results (Table X). It should be noted that these test solutions were neither filtered nor centrifuged prior to extraction and analyses; therefore, total organic constituents (i.e., in solution and adsorbed to particulate matter) were measured for each test phase. This procedure was followed because the toxicity test phases were prepared in a similar manner with test organisms being exposed to similar constituents either adsorbed to the particles or in solution. It should be noted that there was a fivefold decrease in #2 fuel oil-like hydrocarbons in the

TABLE X. ANALYSES OF DRILLING FLUIDS FOR #2 FUEL OIL
Concentrations are in mg/g whole mud (wet weight)

Drilling Fluid	No.2 Fuel Oil
AN31	1.18
MIBLKA51	0.19
SV76	3.59
P1	9.43
P2	2.14
P3	3.98
P4	0.67
P5	1.41
P6	0.10
P7	0.50
P8	0.56
Sept. 4 (Mobile Bay)	2.34

Table XI. CONCENTRATIONS OF #2 FUEL OIL-LIKE HYDROCARBONS IN DRILLING FLUID/SEAWATER TEST PHASES

Drilling Fluid	Type of Phase	Phase Concentration (mL/L)[a]	Concentration (mg/L,ppm)
AN31	Liquid	3.0	0.05
	Suspended	3.0	0.61
MIBLKA51	Liquid	5.0	n.d.
	Suspended	5.0	0.09
SV76	Liquid	5.0	0.07
	Suspended	3.0	2.10
	Sus.(Centrifuge)	3.0	0.41
P1	Liquid	3.0	0.04
	Suspended	1.0	2.55
P2	Liquid	3.0	0.01
	Suspended	0.5	0.24
P3	Liquid	3.0	0.03
	Suspended	1.0	1.23
P4	Liquid	3.0	0.01
	Suspended	0.5	0.10
P5	Liquid	3.0	0.02
	Suspended	0.5	0.13
P6	Liquid	3.0	n.d.
	Suspended	3.0	n.d.
P7	Liquid	3.0	n.d.
	Suspended	3.0	0.07
P8	Liquid	3.0	0.06
	Suspended	3.0	0.36
Sept. 4 (Mobile Bay)	Liquid	1.0	0.01
	Suspended	0.5	1.67
	Liquid	1.0	0.04
	Suspended	0.5	0.87
	Suspended	3.0	0.43

---

[a] Concentrations are mL of whole mud per L of 0.45 um filtered seawater.

n.d. non-detectable.

centrifuged, suspended SV76 sample (continuous centrifugation at 1,000 x G rpm; flow rate 1.0 mL/min) compared to the suspended (non-centrifuged) phase of the same mud (Table XI). Therefore, a major portion of the hydrocarbons in the muds were adsorbed to particles.

GC/MS scans of the suspended phases (1 h settlement) also resembled those of standard API #2 fuel oil. Therefore, #2 fuel oil was used as a standard for quantification of the sample extracts. However, GC/MS scans of the liquid phases (72 h settlment) did not resemble #2 fuel oil. These samples were quantified by using naphthalene as an external standard. GC/MS data from the 1 h settled solution (highest concentation) show various aromatic organic hydrocarbons in the solution. These compounds ranged from 1 to 3 aromatic ring substituted and non-substituted hydrocarbons.

In the 72 h settled solutions, most of the lower molecular weight aromatic compounds were not present. Also, the higher molecular weight alkanes (C_{10} - C_{20} range) were absent. Adsorption, microbial degradation, and volatilization most probably are important factors influencing this decrease of organic constituents over the 72 h settling period.

In order to determine the cause of these decreases, changes in the GC/MS scans of similarly prepared samples of 10 ppm (v/v) #2 fuel oil solution were monitored with time. The liquid samples showed 73% loss of methylene chloride extractable compounds over a 72 h period. The Boston Harbor sea water (0.45 um filtered) used in these experiments may have contained hydrocarbon-utilizing bacteria which contributed to loss of compounds in the 72 h liquid test phases.

Results from the drilling mud-sea water test phase organic analyses have proven to be difficult to interpret since there are many unidentifiable peaks. Adsorption, microbial degradation, and volatilization are three phenomena that may complicate the spectra. Also, tributyl phosphate and acetovanillin (degradation product of lignin) were found in some samples at ppb concentrations.

These previously described phenomena may also contribute to the disparity of concentrations of #2 fuel oil in the liquid phase drilling fluid test solutions compared to concentrations in the whole mud samples. Higher concentrations of hydrocarbons would be expected in the liquid phase because of their relatively high concentrations in the whole mud samples. In particular, the low molecular weight hydrocarbons (earlier eluting compounds) appear to have decreased in concentration in some samples. Exact determination of the carbon chain length of these hydrocarbons was not obtained.

The gas chromatograms of liquid phases of the MIBLKA51, P6, and P7 drilling muds did not show hydrocarbon profiles or other extractable components. This is consistent with the suspended-solids phase data which show low #2 fuel oil concentations for these muds.

IV. SOLID PHASE RECOLONIZATION STUDIES

The recolonization study described in Progress Report #2 (NEA, 1981) was designed to test the effects of a used drilling mud on the recruitment of benthic organisms in defaunated sediment. Data from this study, which involved both a laboratory-based and a field-based experiment, have been analyzed; results were reported in Progress Report #3 (NEA, 1982) and are presented below in their entirety.

4.1 Materials and Methods

The matrices of the laboratory- and field-based experiments were identical and included 15 samples each of three treatments of sediment: a fine-grained reference sediment (Control); a drilling mud mixed with reference sediment (Homogeneous test); and drilling mud deposited on the surface of reference sediment (Surface test). Five replicate samples of each treatment were removed after two weeks, four weeks, and six weeks.

Reference mud consisted of a fine-grained sediment collected from Buzzards Bay, Massachusetts. The reference mud was defaunated by sieving through a 0.5-mm screen and refrigerated until needed. A predetermined weight of reference sediment was added to each container to provide the required volume. The drilling fluid was a medium density lignosulfonate drilling mud supplied by PESA. It was washed by mixing 1 part whole mud with 9 parts sea water. This mixture was thoroughly stirred once an hour for six hours, then allowed to settle for two weeks; the liquid phase was discarded. The volume of the remaining slurry was greater than that of the original whole drilling mud by a factor of 2.4. This factor was used to calculate the volume of washed slurry necessary to provide the required volume of whole drilling mud in each test treatment.

Plastic freezer storage boxes measuring 15.5 cm x 15.5 cm x 10.5 cm high were used as sample containers. Each control sample contained 4 cm of reference sediment. Each homogeneous test sample contained 3.2 cm of reference sediment thoroughly mixed with 0.8 cm of whole drilling mud. Each surface test sample had 0.4 cm of whole drilling fluid deposited on 3.6 cm of reference mud. These two volume ratios were used because they provided a 1:4 ratio of drilling mud to reference sediment in the top 2 cm of substrate, the region in which Woodin (1974) found preferential occupation by five families of polychaetes in a natural mud flat.

For the laboratory-based study, a circular tank 1.9 m in diameter and 0.8 m deep was installed in the laboratory of Northeastern University's Marine Science Institute in Nahant, Massachusetts. This system operated on the principle of passive overflow drainage. Unfiltered sea water, pumped from Massachusetts Bay to holding tanks, was gravity-fed into the center of the tank 21 cm below the surface of the water. A trough attached around the entire circumference of the tank allowed water to drain. This arrangement provided a radially uniform flow of water over the samples containers, which were placed in two rows around the perimeter on the bottom of the tank. The outer row accommodated 27 sample containers, and the inner row 18 sample containers. This experiment was started on April 8, 1981, using the system shown in Figure 4 and the sample arrangement shown in Figure 5.

The field-based study was conducted at the University of Massachusetts Marine Station at Hodgkins Cove, on Cape Ann, Massachusetts. The site has a mixed mud and sand bottom in approximately 7 m of water and is subject to tidal currents of medium strength. Fifteen tightly covered sample containers were placed in a weighted wooden box measuring 0.9 m x 0.6 m x 0.3 m (Figure 6). The box was covered with a sheet of 1-cm mesh plastic grid to prevent intrusions by large predators and strapped to a weighted plastic platform measuring 1.2 m x 1.0 m x 0.2 m. Three such units were lifted by a winch over the side of the 60-ft research vessel "Walter Phipps", sunk and positioned adjacent to each other by SCUBA-equipped divers. Approximately 1.5 hr after emplacement, the covers were removed from the sample containers. This test system (Figure 7) was deployed on April 10, 1981.

Upon collection, samples were sieved through a 0.25 mm screen and preserved in 10% formalin. Each sample was subsequently divided into a 0.5 mm and a 0.25 mm fraction, and stained with Rose Bengal.

The 0.5 mm fraction of each sample was sorted under a stereo dissecting microscope for the two- and four-week recruitment periods of both experiments. Six-week samples were sorted only for the field-based experiment. All animals were

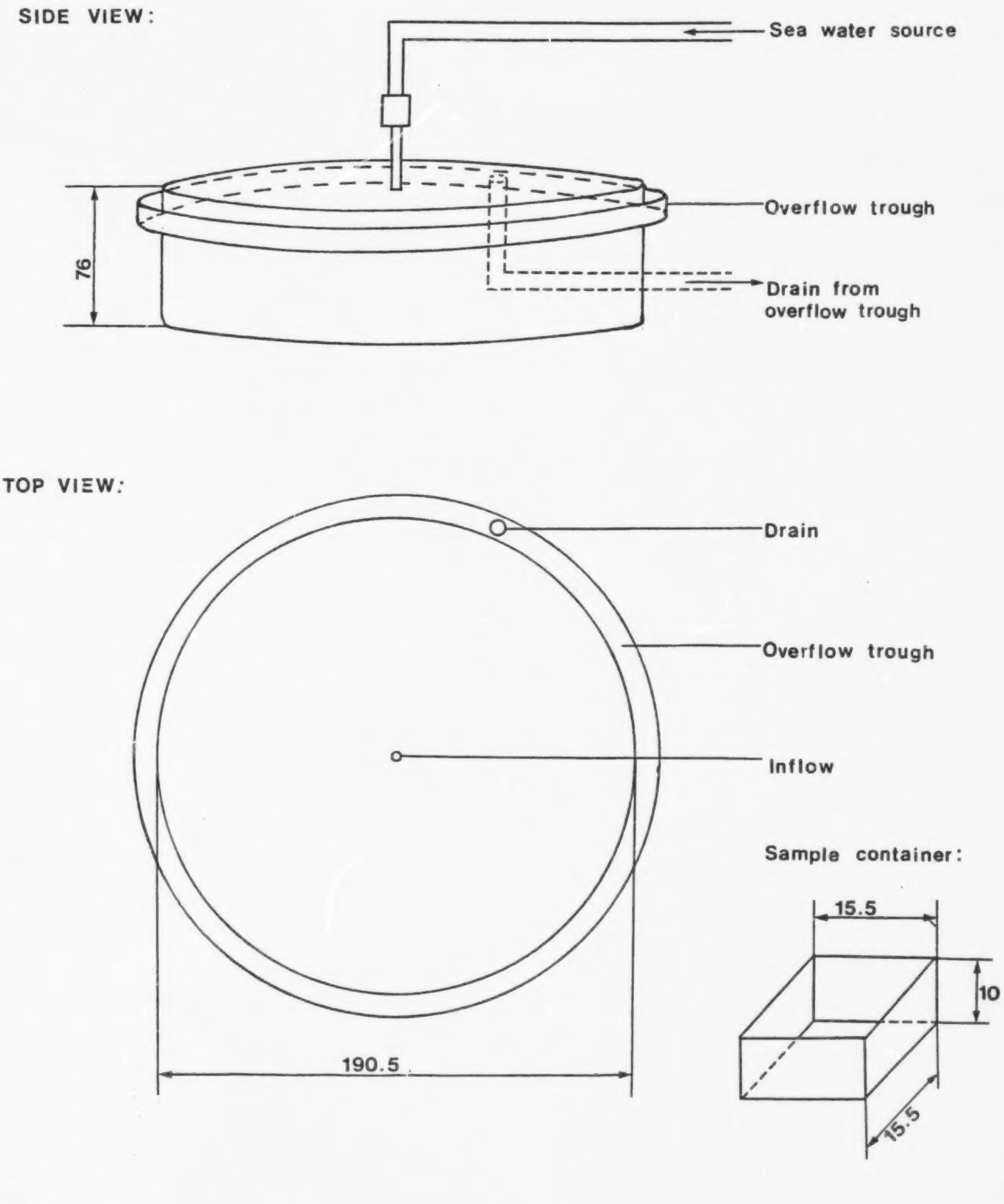

SIDE VIEW:
Sea water source
Overflow trough
76
Drain from overflow trough
TOP VIEW:
Drain
Overflow trough
Inflow
190.5
Sample container:
15.5
10
15.5

FIGURE 4. Laboratory-based Experimental System

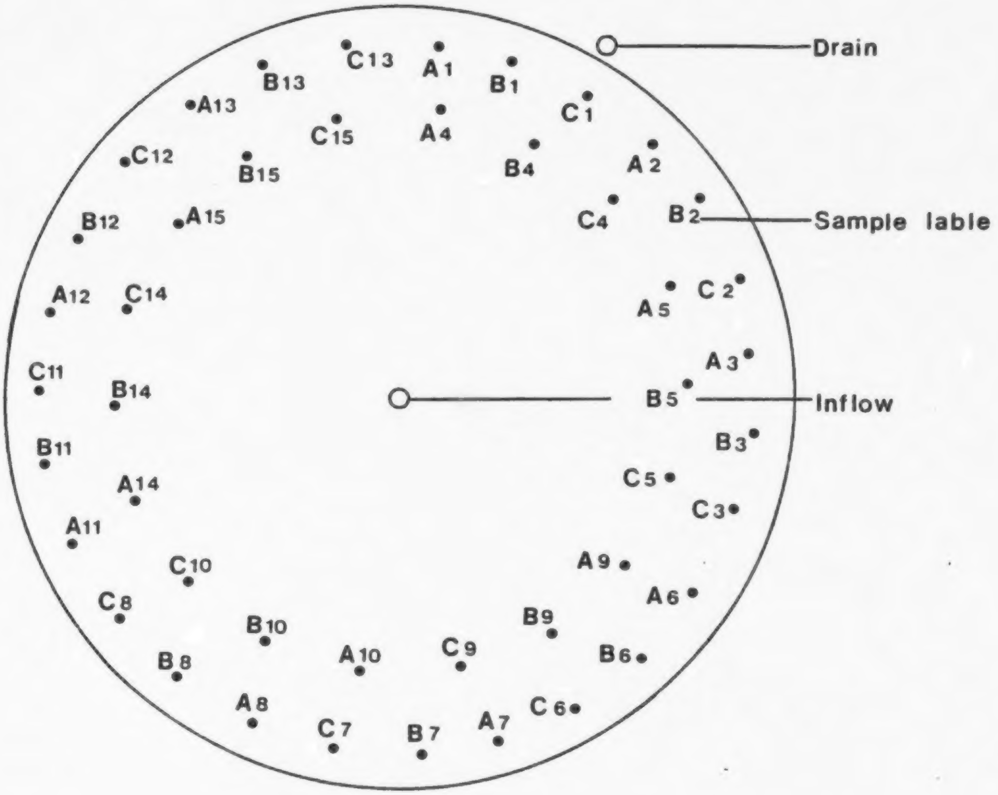

Drain
Sample lable
Inflow
C13
A1
B1
B13
C1
A13
C15
A4
C12
B15
B4
A2
B12
A15
C4
B2
A12
C14
A5
C2
A3
C11
B14
B5
B11
B3
A14
C5
A11
C3
C10
A9
C8
A6
B10
B9
B8
A10
C9
B6
A8
C7
B7
A7
C6

FIGURE 5. Arrangement of Test Containers for Laboratory-based Experiment

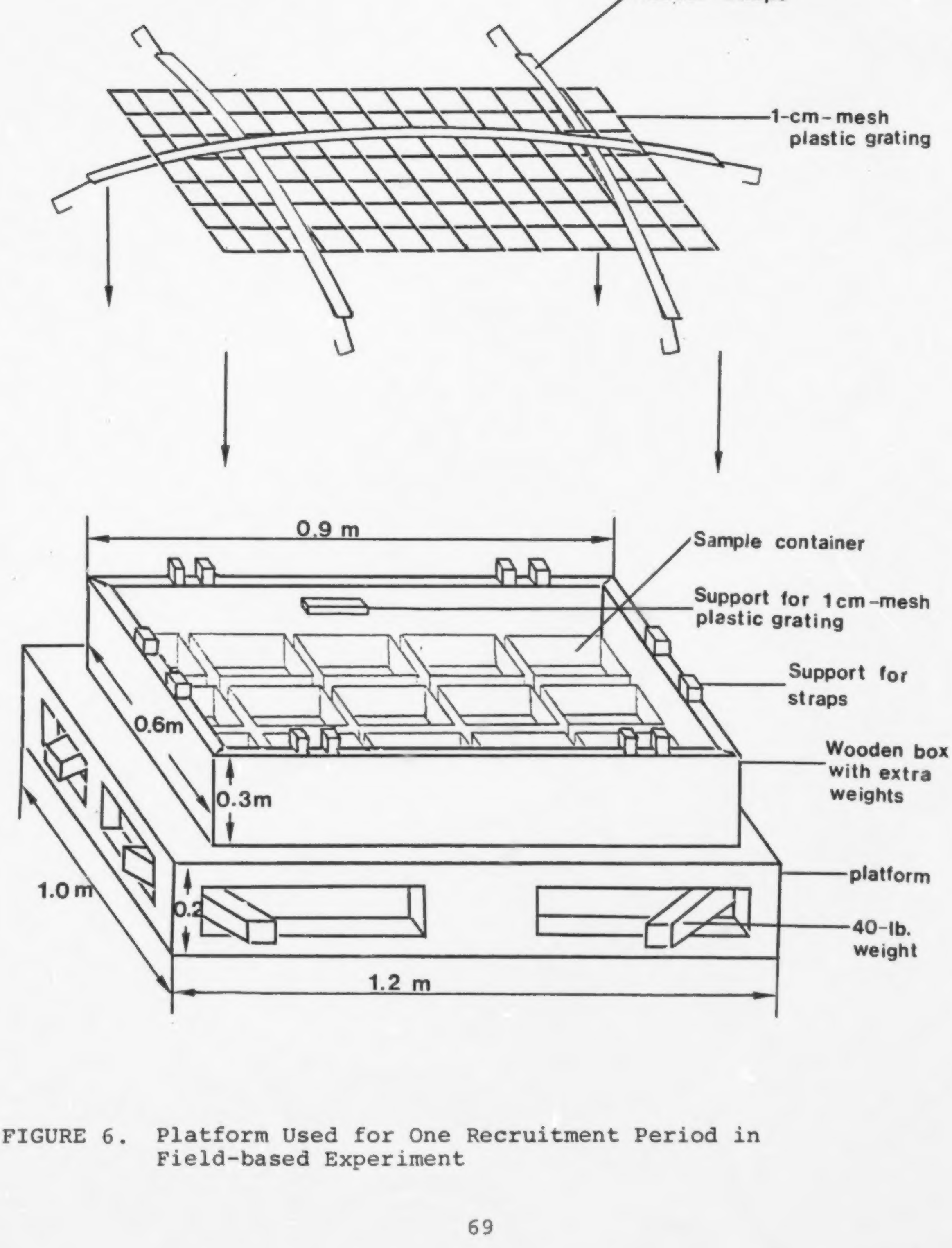
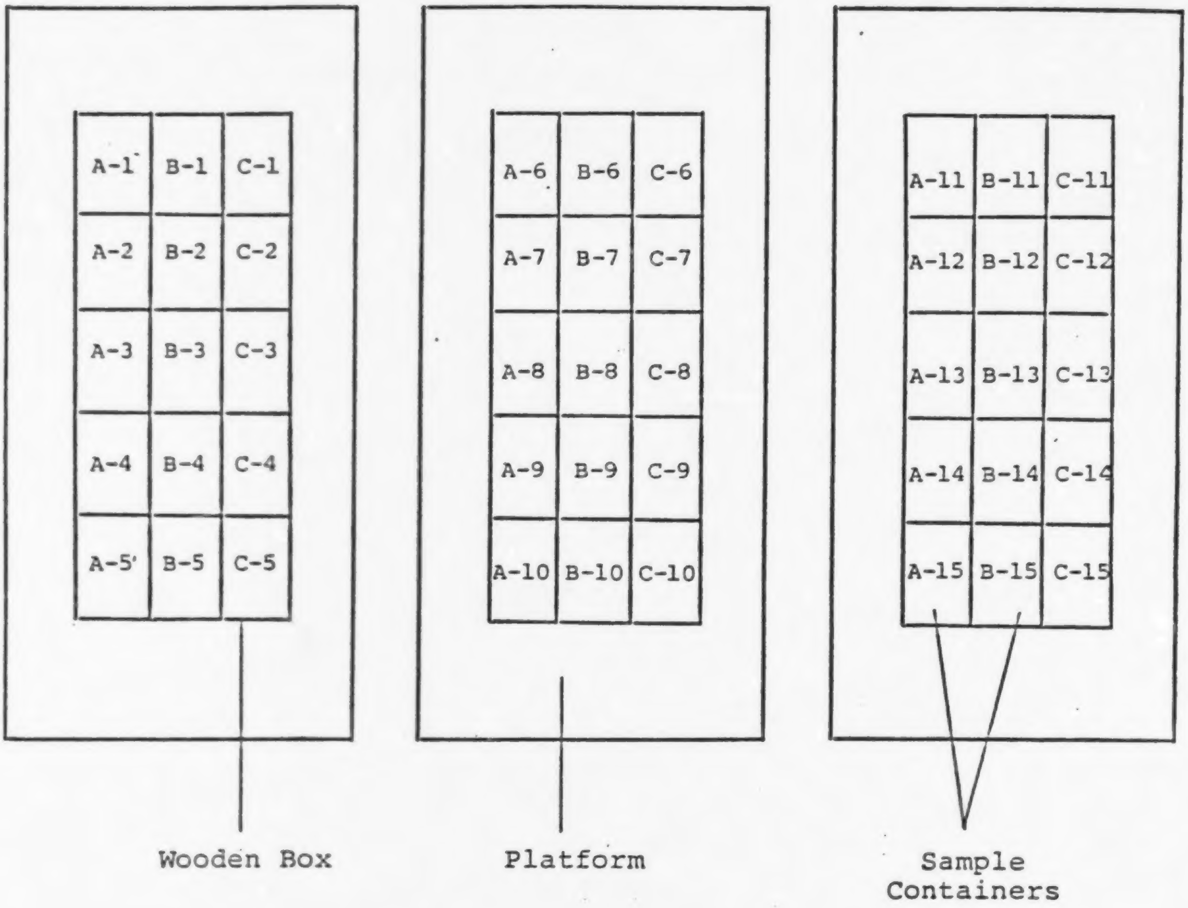

Rubber straps
1-cm-mesh plastic grating
0.9 m
Sample container
Support for 1cm-mesh plastic grating
Support for straps
0.6m
Wooden box with extra weights
0.3m
platform
1.0 m
0.2
40-lb. weight
1.2 m

FIGURE 6. Platform Used for One Recruitment Period in Field-based Experiment


A-1
B-1
C-1
A-2
B-2
C-2
A-3
B-3
C-3
A-4
B-4
C-4
A-5
B-5
C-5
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C-12
A-13
B-13
C-13
A-14
B-14
C-14
A-15
B-15
C-15
Wooden Box
Platform
Sample Containers

Key to Sample Labels

A. Control: Reference Sediment

B. Homogeneous Test: Drilling Mud Mixed with Reference Sediment

C. Surface Test: Drilling Mud Deposited on the Surface of Reference Sediment

1-5: Removed after 2 weeks
6-10: Removed after 4 weeks
11-15: Removed after 6 weeks

FIGURE 7. Arrangement of Test Containers for Field-based Experiment

identified to the lowest possible taxon and counted. Meiofauna (consisting of Nematoda, Arachnida, Ostracoda, and Copepoda) and the planktonic cyprid stage of cirriped larvae were identified and counted but were excluded from data analysis since most members of these taxa were not retained in the 0.5 mm fraction. Colonial species were also excluded.

The 0.25 mm fraction was sorted and animals identified only for the four-week control and surface test samples of the lab-based experiment, in order to determine whether sorting the smaller fraction and including meiofauna had an effect on the results. Data from both fractions, including meiofauna, were combined and compared statistically to those from the 0.5 mm fraction alone, excluding meiofauna.

Three parameters were used for statistical analysis of samples from each recruitment period of each experiment: (1) number of individuals; (2) number of species; and (3) ratio of numbers of species and individuals. The number of individuals indicates the overall abundance in a unit area. The number of species is a measure of variety or species richness. Both of these determine the third parameter, number of species/number of individuals (S/N), which is a simple estimator of diversity, uncorrected for sample size or evenness of species distribution. Analysis of variance and the Student-Newman-Keuls multiple range test were performed to compare the treatments for the above parameters. Student's t-test was used for groups of data in which only two treatments were being compared. In all statistical tests, a 95% confidence level was used ($P \leq 0.05$). Recolonizing populations were also qualitatively characterized by distribution of individuals by phylum and by species predominance. Species were considered "predominant" on the following basis: each predominant species occurred in at least 60% of the replicates and contributed at least 4% of the total animals in a treatment. Less abundant species were also included until 75% of the total animals in a sample were accounted for.

Within each experiment, patterns of community development over time in the three treatments are compared, using percentage change in abundance of all animals and of important taxa between recruitment periods. Results are discussed with reference to similar studies that have been performed. In addition, the lab- and field-based experiments are compared in terms of methodology.

4.2 Results

Appearance of samples at the time of collection was similar in both experiments: each sample was covered with a layer of detritus which increased over time. Detrital deposits were greater in the field-based experiment. In the surface test samples of both experiments, the layer of drilling fluid did not wash out of samples and remained distinct under the detritus.

Mean numbers (± standard deviation) of animals recovered for all samples analyzed in both experiments are displayed in Figure 8. Numbers of animals increased over time in both

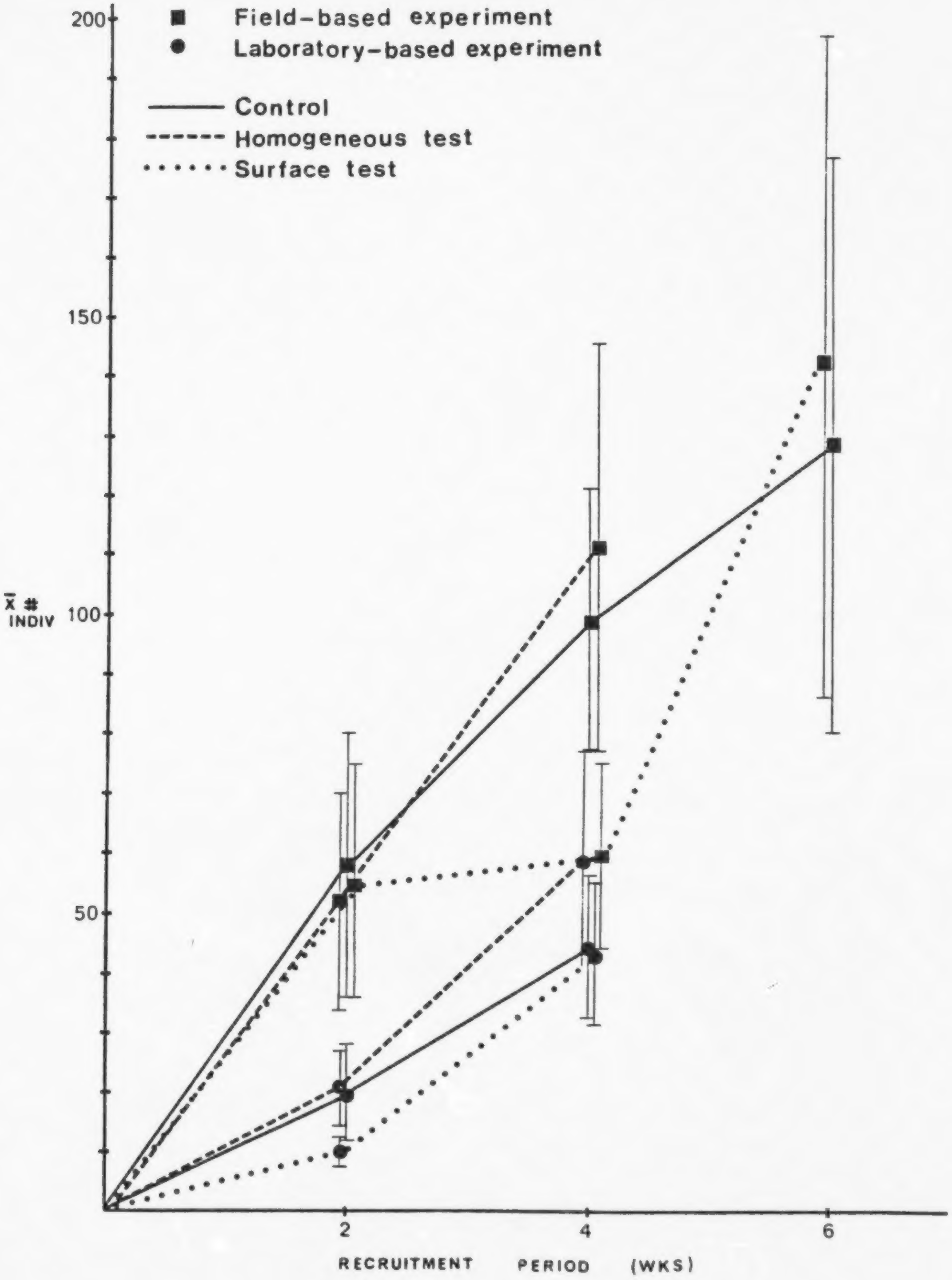

FIGURE 8. Mean number of individuals collected after two-, four-, and six-week recruitment periods for control, homogeneous and surface test treatments in laboratory- and field-based experiments. Data are mean of individuals for n = 5 replicates (except for lab-based two-week homogeneous test, where n = 4). Vertical bars indicate standard deviations.

lab- and field-based experiments, and the latter had a higher number of animals than the former after both two- and four-week recruitment periods.

4.2.1 Laboratory-Based Experiment

4.2.1.1. Two-Week Samples

After two weeks, 231 animals were collected from the lab-based experiment, representing 30 species in five phyla (Table XII). For all treatments combined, the most abundant phylum was Annelida (50% of all animals), followed by Chordata (26%), Arthropoda (16%) and Mollusca (8%). The three predominant species were the polychaete Fabricia sabella, the tunicate Molgula sp., and the tubificid oligochaete Peloscolex benedeni.

The mean number of individuals found in control samples was 19.4 ± 9.4, that for the homogeneous test treatment was 20.8 ± 6.8, and that for surface test was 10.2 ± 2.6 (Table XIII).

The mean number of species for the three treatments were 6.8 ± 2.2 for control, 9.6 ± 2.5 for homogeneous, and 7.0 ± 1.0 for surface test samples. Control, homogeneous, and surface samples had mean S/N ratios of 0.43 ± 0.23, 0.47 ± 0.09, and 0.72 ± 0.18, respectively. Although analysis of variance showed no significant difference between treatments at $P < 0.05$ for any of the three parameters, differences for number of individuals and S/N ratio were significant at $P < 0.10$.

A similarity between treatments was exhibited when faunal distribution by phylum was considered (Table XIV). In all

Table XII: TOTAL NUMBER OF INDIVIDUALS (N), SPECIES (S) AND PHYLA (P) PER TREATMENT AND FOR ALL TREATMENTS COMBINED (5 REPLICATES/TREATMENT)

1a. Laboratory-based Experiments

Recruitment Period	2 WEEKS				4 WEEKS			
	Control	Homog. Test	Surface Test	Treatments Combined	Control	Homog. Test	Surface Test	Treatments Combined
N	97	104+	51	231	219	298	214	731
S	17	19	16	30	23	22	23	29
P	4	4	5	5	4	5	3	6

+ Adjusted for 5 replicates

1b. Field-based Experiments

Recruitment Period	2 WEEKS				4 WEEKS				6 WEEKS		
	Control	Homog. Test	Surface Test	Treatments Combined	Control	Homog. Test	Surface Test	Treatments Combined	Control	Surface Test	Treatments Combined
N	292	260	274	826	495	555	299	1349	645	708	1353
S	34	36	29	49	35	43	32	52	26	31	35
P	3	5	3	5	5	5	4	6	3	4	4

Table XIII:

Number of individuals (N), number of species (S), and diversity index (S/N) for control, homogeneous and surface test treatments in the laboratory- and field-based experiments. Data are mean ± standard deviation (n = 5 replicates/treatment except for two week homogeneous test where n = 4). NA = samples not analyzed.

Experiment	Recruitment Period	Parameter	TREATMENT		
			Control	Homogeneous Test	Surface Test
Laboratory-based	2 WKS	N	19.4 ± 9.4	20.8 ± 6.8	10.2 ± 2.6
		S	6.8 ± 2.2	9.6 ± 2.5	7 ± 1
		S/N	0.43 ± 0.228	0.472 ± 0.094	0.722 ± 0.182
	4 WKS	N	43.8 ± 11.6	59.6 ± 18.1	42.8 ± 12.3
		S	11.6 ± 3.1	10.4 ± 1.9	11 ± 1.9
		S/N	0.277 ± 0.092	0.180 ± 0.033	0.268 ± 0.057
Field-based	2 WKS	N	58.4 ± 22.0	52 ± 18	54.8 ± 20.3
		S	14.2 ± 3.03	15.6 ± 3.8	14.2 ± 2.9
		S/N	0.265 ± 0.079	0.330 ± 0.112	0.271 ± -0.055
	4 WKS	N	99 ± 22.5	111 ± 34.4	59.8 ± 16.4
		S	18.8 ± 2.2	19.8 ± 3.6	17.4 ± 2.7
		S/N	0.200 ± 0.065	0.191 ± 0.55	0.303 ± 0.073
	6 WKS	N	129 ± 48.6	NA	141.6 ± 55.7
		S	13.6 ± 0.89	NA	15 ± 3.5
		S/N	0.114 ± 0.025	NA	0.118 ± 0.045

Table XIV:

Laboratory-based Experiment: Faunal distribution by phylum for control, homogeneous and surface test treatments over two recruitment periods. Data are % contribution by phylum and total number of individuals in 5 replicates (in parentheses)

Recruitment Period	Treatment	PHYLUM				
		Moliusca	Annelida	Arthropoda	Chordata	Other
2 WKS	Control	6.2 (6)	54.6 (53)	10.3 (10)	28.9 (28)	--
	Homog. Test	7.2 (6+)	50.6 (42+)	20.5 (17+)	21.7 (18+)	--
	Surface Test	11.8 (6)	41.2 (21)	15.7 (8)	29.4 (15)	1.9 (1)
4 WKS	Control	5.4 (12)	65.8 (144)	28.3 (62)	--	0.5 (1)
	Homog. Test	6.7 (20)	81.2 (242)	11.4 (34)	--	0.7 (2)
	Surface Test	7.0 (15)	68.2 (146)	24.8 (53)	--	--

+ numbers of individuals in 4 replicates

Table XV:

Laboratory-based Experiment: List of predominant species for control, homogeneous and surface test treatments over two recruitment periods. Occ. = Occurrence per 5 replicates, except in two week homogeneous test, where n = 4

	CONTROL					HOMOGENEOUS TEST					SURFACE TEST				
Recruitment Period	Species	# of Indiv.	Occ.	% of Total	Cumul. %	Species	# of Indiv.	Occ.	% of Total	Cumul. %	Species	# of Indiv.	Occ.	% of Total	Cumul. %
2 WKS	1. Fabricia sabella	32	4	33.0	33.0	1. Fabricia sabella	23	4	27.7	27.7	1. Molgula sp.	15	5	29.4	29.4
	2. Molgula sp.	28	5	28.9	61.7	2. Molgula sp.	18	4	21.7	49.4	2. Fabricia sabella	12	4	23.5	52.9
	3. Peloscolex benedeni	14	3	14.4	76.3	3. Peloscolex benedeni	11	3	13.3	62.7	3. Peloscolex benedeni	5	3	9.8	62.7
						4. Jassa falcata	4	3	4.8	67.5	4. Corophium sp.[b]	3	2	5.8	68.5
						5. Pleusymtes glaber	4	1	4.8	72.3	5. Dexamine thea	2	2	2.4	70.9
						6. Mytilidae	3	2	3.6	75.9	6. Capitellidae[c]	2	2	2.4	73.3
											7. Mytilidae	2	2	2.4	75.7
											8. Naticidae	2	2	2.4	78.1
4 WKS	1. Fabricia sabella	98	5	44.7	44.7	1. Fabricia sabella	208	5	69.8	69.8	1. Fabricia sabella	93	5	43.5	43.5
	2. Peloscolex benedeni	33	5	15.1	59.8	2. Peloscolex benedeni	22	5	7.4	77.2	2. Peloscolex benedeni	38	5	17.8	61.2
	3. Corophium sp.	23	5	10.5	70.3						3. Corophium sp.[b]	16	4	7.5	68.7
	4. Marinogammarus sp.[a]	12	4	5.5	75.8						4. Aoridae	10	3	4.7	73.4
											5. Mytilidae	9	5	4.2	77.6

a - probably Marinogammarus stoerensis

b - including Corophium bonelli and C. crassicorne

c - probably Capitella capitata (see text) treatments, Annelida was the most abundantly represented phylum, followed in decreasing order by Chordata, Arthropoda, and Mollusca.

In each of the three treatments, the same three species were predominant (Table XV). Although surface test samples showed a slightly different order of predominance, overall percentages of abundance were very similar for the three treatments.

4.2.1.2 Four-Week Samples

A total of 731 animals was collected after four weeks, representing 29 species in six phyla (Table XII). Considering all treatments together, annelids were predominant (73% of fauna), followed by arthropods and molluscs (20% and 6%, respectively). The most abundant species were the annelids Fabricia sabella and Peloscolex benedeni, the amphipod Corophium sp. (including C. bonelli and C. crassicorne), and juveniles of a Mytilid mussel.

The mean number of individuals found in control samples was 43.8 ± 11.6. The mean for homogeneous test samples was 59.6 ± 18.1, and the mean for surface samples was 42.8 ± 12.3 (Table XIII). Mean numbers of species were 11.6 ± 3.1, 10.4 ± 1.9, and 11.0 ± 1.9, respectively, for control, homogeneous and surface test samples, and the three treatments had mean S/N ratios of 0.28 ± 0.09, 0.18 ± 0.03, and 0.27 ± 0.06 respectively. Treatments were not significantly different for any of the three parameters (ANOVA, $P > 0.05$).

When data from the 0.5 mm and 0.25 mm fractions were combined for each control and surface test sample and meiofauna were included, mean numbers of individuals were 186.5 ± 46.9 and 186.5 ± 26.8, respectively, for the two treatments. Corresponding mean numbers of species were 18.5 ± 3.1 and 17.0 ± 0.8; mean S/N ratios were 0.10 ± 0.02 and 0.09 ± 0.01. Performance of Student's t-test showed no significant difference between the two means for any parameter ($P > 0.05$). These results agreed with results obtained from the 0.5 mm fraction alone, with meiofauna excluded.

Considering distribution of animals by phylum, annelids were most abundant in each treatment, followed by arthropods and molluscs (Table XIV). The percentage distribution was somewhat different for homogeneous test samples: annelids accounted for a greater percentage of fauna found in this treatment, and the other two phyla comprised correspondingly lower percentages.

In each of the treatments, Fabricia sabella was the predominant species although by a higher percentage in the homogeneous test samples (70%, as compared with 45% and 44%, respectively, for control and surface samples). (See Table XV.) Second in abundance in each treatment was Peloscolex benedeni.

4.2.1.3 Comparison of Recruitment Periods

Surface test samples exhibited the highest percentage of growth, increasing 320% from 51 to 214 individuals (Figure 9a). The number of animals recovered in homogeneous samples increased

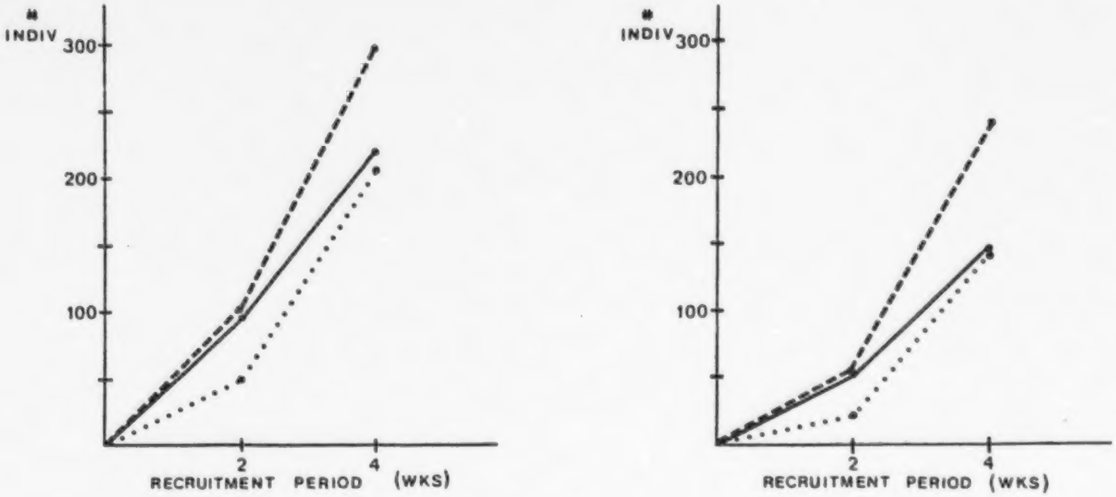

FIG. 9a. All Taxa

FIG. 9b. Annelida

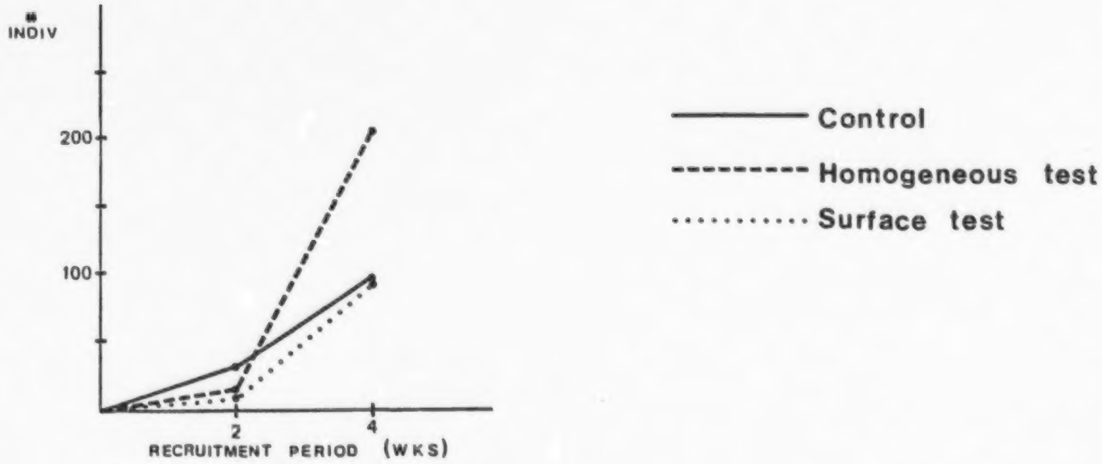

FIG. 9c. Fabricia sabella

FIGURE 9. Laboratory-based Experiment: Total Number of Individuals for Important Taxa in Control, Homogeneous and Surface Test Treatments Over Two Recruitment Periods. (Numbers are pooled data from 5 replicates except for two week homogeneous test, where data from 4 replicates are adjusted for comparison).

187% from 104 to 298 animals. Control samples displayed the smallest increase: 126%, from 97 animals after two weeks to 219 individuals after four weeks.

The phylum Annelida remained predominant and contributed more than any other phylum to the increase in number of animals (Figure 9b). Surface samples contained 595% more annelids after four weeks than after two weeks. Corresponding percentages for homogeneous and control samples were 357% and 172%, respectively.

The annelid species which accounted for this increase was _Fabricia sabella_ (Figure 9c). There were over six times more _F. sabella_ in the four-week sampling of both homogeneous and surface test samples. Control samples tripled in number of this species. The oligochaete _Peloscolex benedeni_ also increased in all treatments.

The tunicate _Molgula_ sp., which occurred in all two-week samples and represented 26% of the fauna, was not present in the four-week samples. This disappearance accounted for corresponding increases in predominance of other species and phyla between the two recruitment periods. No other changes occurred in the order of species predominance or relative distribution by phylum from the two-week to the four-week samples.

4.2.2 Field-Based Experiment

4.2.2.1 Two-Week Samples

After two weeks, 826 animals belonging to 48 species in five phyla, were recovered from the field-based experiment

(Table XII). Combining all treatments, Arthropoda was by far the predominant phylum, constituting 72% of total individuals, followed by Annelida (21%) and Mollusca (7%). Recently metamorphosed adults of a cirriped barnacle were the most abundant species. Other predominant species were the amphipod Marinogammarus sp. (probably Marinogammarus stoerensis), the isopod Edotea montosa and the polychaete Harmathoe sp.

Control contained samples had a mean number of individuals of 58.4 ± 22.0, which was similar to those for test samples (52.0 ± 18.0 and 54.8 ± 20.3 for homogeneous and surface samples; see Table XIII). Mean numbers of species for control, homogeneous and surface test samples were 14.2 ± 3.0, 15.6 ± 3.8, and 14.2 ± 2.9, respectively. Mean S/N ratios were 0.27 ± 0.08, 0.33 ± 0.11, and 0.27 ± 0.06 for the three treatments, respectively. Analysis of variance showed no significant difference between treatments for any of these parameters.

Distribution of animals by phylum was the same in each treatment: arthropods were considerably more abundant than annelids and molluscs (Table XVI). The same four species predominated in all three treatments (Table XVII). The order of predominance was identical for control and homogeneous test samples, and quite similar for surface samples, although percentage contribution by the predominant cirriped barnacle in the latter was lower than in the other two treatments.

Table XVI:

Field-based Experiment: Faunal distribution by phylum for control, homogeneous and surface test treatments over three recruitment periods. Data are % contribution by phylum and total number of individuals in 5 replicates (in parentheses).

Recruitment Period	Treatment	PHYLUM			
		Mollusca	Annelida	Arthropoda	Other
2 WKS	Control	7.9 (23)	22.2 (65)	69.9 (204)	--
	Homog. Test	5.4 (14)	20.0 (52)	73.1 (190)	1.5 (4)
	Surface Test	7.7 (21)	20.1 (55)	72.2 (198)	--
4 WKS	Control	24.6 (122)	30.3 (150)	44.4 (220)	0.6 (3)
	Homog. Test	18.7 (104)	36.9 (205)	43.8 (243)	0.5 (3)
	Surface Test	27.1 (81)	28.1 (84)	44.5 (133)	0.3 (1)
6 WKS*	Control	22.5 (145)	62.8 (405)	14.7 (95)	--
	Surface Test	20.3 (144)	62.1 (440)	17.1 (121)	0.4 (3)

*Homogeneous test samples not analyzed

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Table XVII:

Field-based Experiment: List of predominant species for control, homogeneous and surface test treatments over three recruitment periods. Occ. = Occurrence per 5 replicates.

	CONTROL					HOMOGENEOUS TEST					SURFACE TEST				
Recruitment Period	Species	# of Indiv.	Occ.	% of Total	Cumul. %	Species	# of Indiv.	Occ.	% of Total	Cumul. %	Species	# of Indiv.	Occ.	% of Total	Cumul. %
2 WKS	1. Cirripedia	88	5	30.1	30.1	1. Cirripedia	81	5	31.2	31.2	1. Cirripedia	64	5	23.7	23.7
	2. Marinogammarus sp.^{e}	39	5	13.4	43.5	2. Marinogammarus sp.^{e}	43	5	16.5	47.7	2. Edotea montosa	50	5	18.2	41.9
	3. Edotea montosa	29	5	9.9	53.4	3. Edotea montosa	25	5	9.6	57.3	3. Marinogammarus sp.^{e}	46	5	16.8	58.7
	4. Harmathoë sp.	26	5	8.9	62.3	4. Harmathoë sp.	20	5	7.7	65.0	4. Harmathoë sp.	25	4	9.1	67.8
	5. Calliopius laeviusculus	13	3	5.5	67.8	5. Capitellidae^{a}	11	4	4.2	69.2	5. Capitellidae^{a}	15	5	5.5	73.3
	6. Capitellidae^{a}	14	4	4.8	72.6	6. Calliopius laeviusculus	10	4	3.8	73.0	6. Tellinidae	14	4	5.1	78.4
	7. Tellinidae	14	3	4.8	77.4	7. Tellinidae	8	5	2.4	75.4	7. Corophium sp.^{b}	11	4	4.0	82.4
						8. Corophium sp.^{b}	8	5	2.4	77.8					
4 WKS	1. Tellinidae	108	5	21.8	21.8	1. Cirripedia	122	5	22.0	22.0	1. Tellinidae	72	5	24.1	24.1
	2. Capitellidae^{a}	87	5	17.6	39.4	2. Tellinidae	96	5	17.3	39.3	2. Cirripedia	49	5	16.4	40.5
	3. Edotea montosa	60	5	12.1	51.5	3. Capitellidae^{a}	59	5	10.6	49.9	3. Capitellidae^{a}	38	5	12.7	53.2
	4. Cirripedia	58	5	11.7	63.2	4. Edotea montosa	49	5	8.8	58.7	4. Edotea montosa	22	5	7.4	60.6
	5. Harmathoë sp.	35	5	7.1	70.3	5. Harmathoë sp.	48	5	8.6	67.3	5. Harmathoë sp.	20	5	6.7	67.3
	6. Marinogammarus sp.^{e}	29	5	5.9	76.2	6. Polydora sp.^{c}	48	5	8.6	75.9	6. Polydora sp.^{c}	11	5	3.7	71.0
											7. Corophium sp.^{b}	10	4	3.3	74.3
											8. Marinogammarus sp.^{e}	10	4	3.3	77.6
6 WKS	1. Capitellidae^{a}	344	5	53.3	53.3						1. Capitellidae^{a}	327	5	46.2	46.2
	2. Tellinidae	131	5	20.3	73.6						2. Tellinidae	139	5	19.6	65.8
	3. Edotea montosa	63	5	9.8	83.4						3. Edotea montosa	61	5	8.6	74.4
											4. Polydora sp.^{c}	54	4	7.6	82.0

a - probably Capitella capitata (see text)
b - includes Corophium bonelli and C. crassicorne
c - includes Polydora ligni
d - probably Tellina agilis
e - probably Marinogammarus stoerensis

4.2.2.2 Four-Week Samples

Total number of animals recovered after four weeks was 1349, representing 52 species in six phyla (Table XII). Arthropoda was the most abundant phylum in all treatments combined (44% of total recovery); next were Annelida (33%) and Mollusca (23%). The predominant species was a member of the pelecypod family Tellinidae, which was probably _Tellina agilis_ but could not be positively identified. Cirripeds were next in abundance, followed by a capitellid polychaete. The latter taxon includes animals definitely identified as one of the sibling species of _Capitella capitata_, as well as younger animals which could be assigned only tentatively to this genus. The isopod _Edotea montosa_ and the polychaete _Harmathoe_ sp. were also among the most abundant species.

Mean numbers of individuals were similar for control and homogeneous test samples: 99 ± 22.5 and 111.0 ± 34.4, respectively (Table XIII). In surface samples, 59.8 ± 16.4 individuals were recovered. Mean number of species, similar for all treatments, were 18.8 ± 2.2, 19.8 ± 3.6, and 17.4 ± 2.7 species, respectively. Mean S/N values were 0.20 ± 0.07 for control, 0.19 ± 0.06 for homogeneous, and 0.30 ± 0.07 for surface test samples. A significant difference between treatments for the parameters number of individuals and S/N was revealed by analysis of variance ($P < 0.05$). Student-Newman-Keuls multiple range test showed this to be due to a difference between homogeneous and surface test treatments.

Arthropods were the predominant phylum, accounting for 44% of the fauna in each treatment (Table XVI). Annelids and molluscs were next in abundance in each treatment. The percentage contribution by Annelida was higher in homogeneous test samples than in either of the other treatments.

In each treatment, the same four species were most abundant, but their order of predominance varied (Table XVII). In control samples, tellinids were most abundant, followed in decreasing order by capitellids, *Edotea montosa*, and cirripeds. In both test treatments, cirripeds were relatively more abundant than in the control treatment: in homogeneous samples they were the predominant species, and in surface samples they were second in abundance. The other three species mentioned above remain in the same relative order of abundance as in control samples.

4.2.2.3 Six-Week Samples

Since results of four-week samples indicated a depressed recovery in surface test samples, it was decided to analyze six-week samples for this treatment and compare them to those of the control treatment. Homogeneous test samples were not analyzed. A total of 1353 individuals were recovered in the two treatments, representing 35 species in four phyla (Table XII).

The mean number of individuals for control samples was 129.0 ± 48.6, while that for surface test samples was 141.6 ± 55.7 (Table XIII). Mean numbers of species were 13.6 ± 0.9 and

15.0 ± 3.5, and S/N values were 0.11 ± 0.03 and 0.12 ± 0.05, for control and surface samples, respectively. Student's t-test showed no significant ($P > 0.05$) difference between the treatments for any parameter.

The two treatments resembled each other when considering distribution of animals by phylum: annelids predominated, followed by molluscs and arthropods. The treatments also showed species predominance by the same three species, each contributing similar percentages of the total number of individuals.

4.2.2.4 Comparison of Recruitment Periods

Homogeneous test samples showed the highest rate of growth between two-week and four-week samples, increasing 114% from 260 to 555 individuals (Figure 10a). Control samples increased 70% from 292 to 495 animals. In surface samples, 274 individuals were recovered after two weeks, and only 9% more after four weeks (299 animals). The much slower rate of increase in surface samples was distributed across almost all species and phyla.

Arthropoda was the predominant phylum after both two and four weeks, although other phyla exhibited more substantial increases in number between the two periods. Control and homogeneous test samples each contained a higher number of arthropods after four weeks than two weeks (8% and 28% increases, respectively; see Figure 10b). On the contrary, surface samples showed a 32% decrease in arthropod recovery.

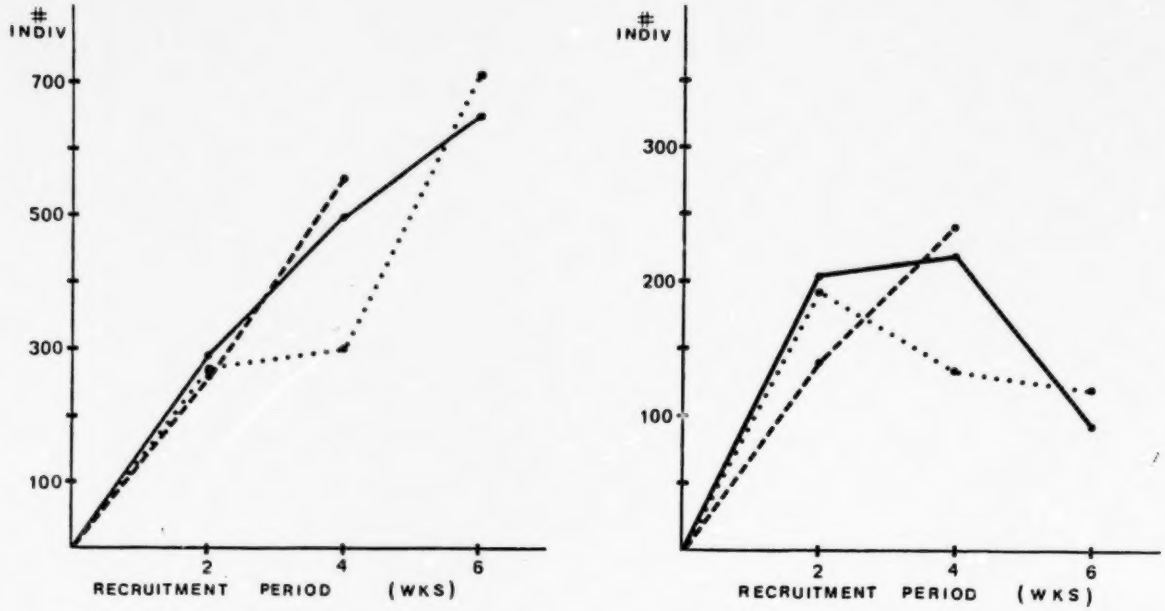

FIG. 10a **All Taxa**

FIG. 10b **Arthropoda**

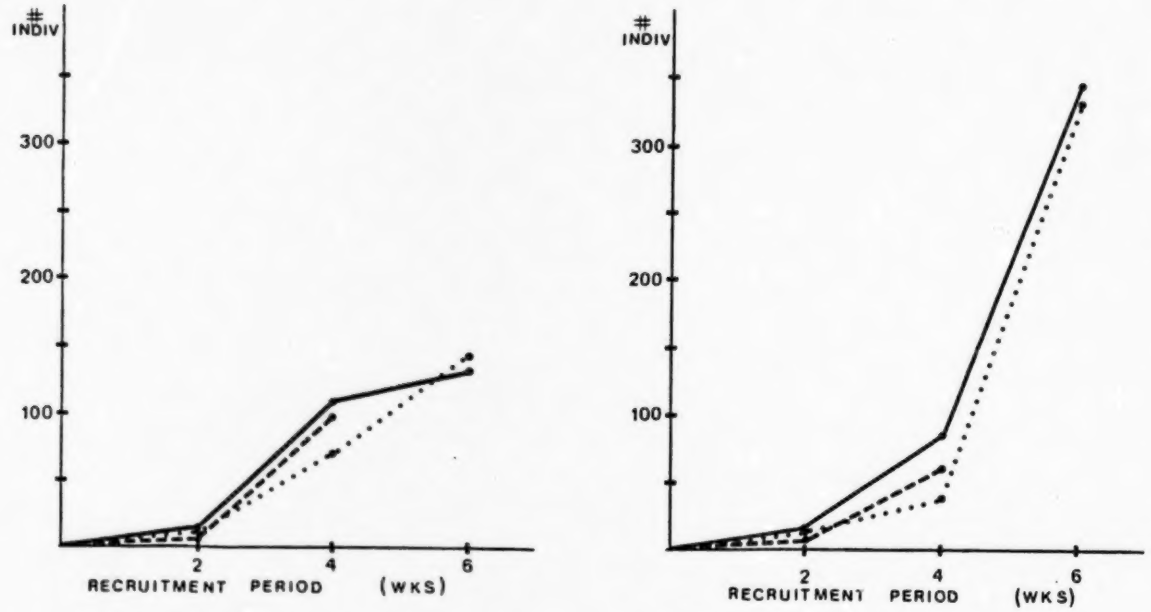

FIG.10c **Tellinidae**

FIG.10d **Capitellidae**

———— Control
- - - - - - - - Homogeneous test
· · · · · · · · · Surface test

FIGURE 10. Field-based Experiment: Total Number of Individuals for Important Taxa in Control, Homogeneous and Surface Test Treatments Over Three Recruitment Periods. (Numbers are pooled data from 5 replicates).

Compared with the other two treatments, surface test samples also displayed the smallest percentage increase in number of annelids and molluscs. Annelid recovery increased 131% in control and 294% in homogeneous samples, but only 53% in the surface treatment. Corresponding percentages of increase for the phylum Mollusca were 430%, 643%, and 190% for control, homogeneous, and surface samples, respectively.

Shifts in the predominant species between the two recruitment periods reveal five species whose trends of abundance are of interest (Table XVII). The cirriped barnacle, which was most abundant in all three treatments after two weeks, decreased by about 30% in control and surface test samples, but increased by 50% in homogeneous test samples after four weeks. The amphipod Marinogammarus sp., second in abundance in all treatments after two weeks, decreased by at least 25% in all treatments and was no longer predominant after four weeks. The isopod Edotea montosa decreased by 56% in surface samples but doubled in the other two treatments.

Two species exhibited significant increases in abundance in all three treatments. Tellinids and capitellids each accounted for around 5% of the total fauna in each treatment after two weeks (Table XVII). By the time of the four-week sampling, the numbers of tellinids recovered had jumped by 11 times in homogeneous test, over 6 times in control and 4 times in surface samples (Figure 10c). In the four-week samples, tellinids were the most abundant species in control and surface test samples (22% and 24% of all animals, respectively) and second in abundance in homogeneous samples (17% of faunal recovery). The rise in number of capitellids over the same time period was most dramatic in control samples (521%), almost as high in homogeneous samples (436%), and smallest in surface samples (153%; see Figure 10d). After four weeks, capitellids were second in predominance in control samples (representing 18% of faunal recovery), and the third most abundant species in homogeneous and surface samples (11% and 13% of all animals in each treatment, respectively).

Control and surface test samples from the six week recruitment period revealed a continuation of patterns of species distribution, but a reversal in numerical trends. Control samples increased 30% in faunal recovery while surface samples increased by 137% between four- and six-week samples (Figure 10a). This escalation compensated for the depressed recovery in surface samples after four weeks: after six weeks, the numbers of animals recovered in the two treatments were very close (645 animals in control and 708 animals in the surface samples).

Predominance in the two treatments after six weeks was very similar. A shift occurred in relative predominance by phylum between the four- and six-week periods (Table XVI). Annelida was by far the predominant phylum after six weeks, comprising 62% of total recovery in each treatment. Mollusca was next in abundance (around 21% for each treatment), followed by Arthropoda (around 16% for each treatment). Capitellids and tellinids continued to increase and constituted, respectively, about 50% and 20% of all animals in each treatment (Table XVI).

4.3 Discussion

The data used for statistical analysis often exhibited a large variability between replicates, making interpretation of the results difficult. Consequently, although a 95% confidence level was used to signify statistical significance, the finding of a difference between treatments at the $P < 0.10$ level for lab-based two-week samples deserves further investigation.

The mean number of individuals found after two weeks in lab-based surface test samples was approximately half that of control and homogeneous samples. The depressed recovery in surface samples was more obvious in the phylum Annelida than in other phyla, and was reflected in the smaller number of the predominant species *Fabricia sabella* and *Peloscolex benedeni*. Both depend in some manner on the substrate.

Fabricia sabella is a tube-dwelling sabellid polychaete that feeds by beating cilia on its branchial crown and straining the resulting current of water. Small particles are ingested and the organic material used as food; medium-sized particles are used for tube-building. Depressed numbers of *F. sabella* in surface test samples might have resulted from three factors: a shortage of particles large enough for tube-building; a reduced supply of organic material for nutrition; and a clogging of the branchiae by very fine particles of drilling mud, limiting food ingestion.

Oligochaetes similarly depend on small particles of organic material for food. Most ingest these along with sediment in the course of burrowing; some graze off larger particles in the substrate such as sand or rock. In either case, the oligochaete Peloscolex benedeni might have suffered from a shortage of food in surface test samples.

Any of these deleterious effects would be expected to disappear over time due to the accumulation of detrital material settling from the incoming water. By the time of the four-week sampling in the lab-based experiment, numbers of these species in surface samples resembled those in control samples.

After four weeks, the mean number of individuals in homogeneous test samples was nearly 50% higher than those of control and surface samples. This was reflected in the number of Fabricia sabella in homogeneous samples, which was more than two times that of either of the other treatments. It is unclear which property of the homogeneous treatment accounted for its ability to support such a higher number of this species.

In the field-based experiment, the populations that had recolonized each treatment were indistinguishable in size and composition after two weeks. After four weeks, statistically significant differences existed between the high number of individuals in homogeneous test samples and the low number in surface samples. The control samples supported a 65% larger population than the surface test samples (not statistically significant at $P < 0.05$), and an 11% smaller population than the homogeneous treatment.

The difference in number of individuals between control and homogeneous test samples after four weeks can be accounted for by the recovery of over twice as many cirriped barnacles in homogeneous samples. An increase of cirripeds between the two- and four-week periods occurred only in this treatment. If the more heterogeneous grain size in homogeneous test samples is in some manner responsible for this enhancement, it is unclear why it did not develop after the two-week recruitment period.

The higher percentage contribution by Annelida in homogeneous samples might be explained by the physical nature of the substrate. The mixture of reference mud and drilling mud had less of a tendency to pack together than reference mud alone and this might have facilitated burrowing by annelids.

A depressed number of animals found in surface test samples was observed in all phyla. A combination of factors may explain the finding of a lower recovery in this treatment after four weeks but not after two weeks: increased predominance by species that depend more directly on the substrate, a reduced rate of increase of some species, and mortality in other species.

Recolonization of the defaunated sediment in this experiment presumably occurred by two mechanisms: the settling of planktonic larval stages and the immigration of adults from the surrounding substrate via crawling or suspension by currents. A count of the population after a period of time reflects the number of larval stages that have settled and survived, and the number of adults that have immigrated and survived. Larvae have been shown to be capable of discriminating between potential substrates and delaying metamorphosis until a suitable substrate is found (Thorson, 1966). Survival of both metamorphosed larvae and immigrated adults depends on factors such as the availability of food, the nature of the substrate, and the general quality of the environment. Suppressed numbers in surface test samples could be due to reduced settlement by larvae or greater mortality of all animals in this treatment.

The predominant species after two weeks was a cirriped barnacle, a filter-feeder which lives on the surface of the substrate. Like the polychaete _Fabricia_ _sabella_, cirripeds depend on straining a current of water for procurement of food and probably for gas exchange. Suppression of the population of cirripeds in surface samples might have been expected to occur due to clogging of branchiae by very fine particles of drilling fluid, as observed with _F_. _sabella_ in the lab-based experiment. However, surface samples supported nearly as many cirripeds as each of the other two treatments, providing evidence that potential suffocation by drilling fluid did not cause significant mortality in this species. It might have been prevented by the layer of detritus deposited by the water column. The layer accumulated at a much faster rate in the field-based experiment than in the laboratory system.

In the period between collection of two- and four-week samples the population composition changed: numbers of a capitellid polychaete and a tellinid pelecypod grew rapidly. These are both deposit feeders, which ingest and rework the sediment. The rate of increase of both species was higher in control and homogeneous samples than in surface samples, which could reflect the interaction of these species with the subsurface layer of drilling mud in the latter treatment.

The isopod *Edotea montosa* and the polychaete *Harmathoe* sp., two species which are predominant in the four-week samples, increased between two- and four-week periods in control and homogeneous samples, but declined in surface samples. Since no other species were observed to "bloom" over the same period, these decreases suggest that the surface test treatment was unable to support the original recolonizing populations of these two species, resulting in some mortality.

It is believed that the lower recovery of individuals in surface test samples after four weeks was caused by a combination of the factors discussed above. Any effects by the surface layer of drilling fluid on the recruited population disappeared after six weeks, as shown by the strong resemblance between the two treatments in population size and composition.

Two explanations could account for the relative increase of animals between four and six weeks in surface samples and control samples: either animals moved into the layer of drilling fluid in surface samples, or the detrital deposition had finally accumulated a layer thick enough to act as a new substrate. Since the drilling fluid had such a distinct effect after four weeks, the second case is believed to be more probable. The layer of deposited material included detritus, sand, and smaller inorganic particles swept up from the surrounding bottom, and appeared to be suitable as a substrate.

The effect of suppressing numbers of individuals found in surface samples could have been caused by physical or chemical aspects of the drilling fluid. Three pieces of evidence suggest a physical mechanism. First, if the effect was chemical, adverse effects would be expected to occur to a lesser extent in the homogeneous test samples. This was not the case. In fact, when homogeneous samples differed from the other two treatments, they contained slightly higher numbers of animals. Secondly, the effect ceased when animals were no longer in direct contact with the layer of drilling fluid, yet chemical effects would probably have persisted since toxicants could continue to leach out of the drilling fluid. Finally, organic and trace metal analyses of this particular PESA drilling fluid and liquid phase toxicity testing showed this mud to have a relatively low toxicity.

Barite, a non-toxic weighting agent, is a major component of drilling fluids. Cantelmo _et_ _al_ (1979) found that barite mixed with a sand substrate enhanced the population density of meiofauna, presumably because of increased sediment heterogeneity, but that a cover of barite over sand significantly decreased meiofaunal population density. Tagatz and Tobia (1978) found adverse effects on macrofauna in developing communities after ten weeks when barite either covered a sand substrate, or was mixed in a ratio of 1 part barite to 3 parts sand. Both of these authors suggested that since barite is non-toxic to many marine organisms, the effect of barite is due to its changing of the sediment granulometry.

Tagatz et al (1978) tested the effects of a used lignosulfonate drilling mud on recolonization over a period of eight weeks. Their results showed considerably more pronounced adverse effects of drilling fluid than the data presented here. There are at least two possible explanations for this apparent discrepancy. Sand was used as a reference substrate by Tagatz et al., while our study used a natural, defaunated sediment. The water supply was pumped from a sandy-bottom environment and probably contained larvae "searching" for a sand substrate. The change in grain size caused by the addition of drilling fluid to sand was presumably much more extreme than that caused by its addition to a fine-grained reference mud; the adverse effects might have been more pronounced as a consequence. Another possibility is that the used drilling fluid tested by Tagatz et al contained more toxic components than the PESA mud used in the present study.

In general, these data show that a used PESA drilling fluid affected recolonization when layered on top of defaunated sediment, but not when mixed with it. In both experiments, deposition of a new layer of material on top of the drilling fluid seemed to reduce or reverse the effects, and by four to six weeks after the beginning of the experiment, effects were no longer obvious.

4.4 Evaluation of Methodology for Solid Phase Recolonization Tests

The laboratory-based experiment required five months of preliminary work (described in Progress Report #2; New England Aquarium (1981)) to ensure that the system provided a uniform flow of water over all sample containers. Once this condition had been satisfied, the system offered easy access for deployment and retrieval of samples. Minor maintenance was required every other day during the course of the experiment.

The field-based system required three days of preliminary work in the laboratory to prepare the experimental equipment. Deployment of the samples was fairly easy when using a research vessel equipped with a winch. Two divers were required to assist during deployment and to retrieve samples every two weeks; these operations were therefore limited by the weather. In this experiment, only extremely stormy conditions would have prevented access, since the site was very close to the dock where the divers entered the water.

More animals were collected in the field-based than the lab-based experiment after both two and four weeks of recruitment time. This may have been due to the different locations used for this study. Since a difference between control and test treatments would presumably be more obvious with a larger number of animals in each treatment, the field-based system appeared preferable to the lab-based study.

From January through March, low water temperatures would prohibit use of the field-based system in northern latitudes, while experiments could still be conducted under laboratory conditions. However, planktonic larvae occur in very low numbers in the water column during these months, so there is little advantage to running a recolonization experiment during the winter in temperate climates. In general, it is felt that the amount of time and/or maintenance required to ensure unbiased water flow in a laboratory-based system makes this a less attractive alternative than a field-based system.

Recolonization studies are an improvement on solid phase toxicity tests as a method of assessing the impact of contaminated sediment on the benthic environment. The study described above was a more sensitive measure of the effects of releasing drilling mud, because it considered development of benthic communities rather than the ability of a contaminant to kill adult animals over a ten-day test period (see discussion of solid phase toxicity tests in NEA, 1980). Although the method is a valid approach, we have concluded, based on the present study, that it requires too much time for efficient evaluation of whole drilling muds.

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